XV International AIDS Conference

Pathogenic Mechanisms of HIV Disease: The Role of Viral Replication and Immune Activation

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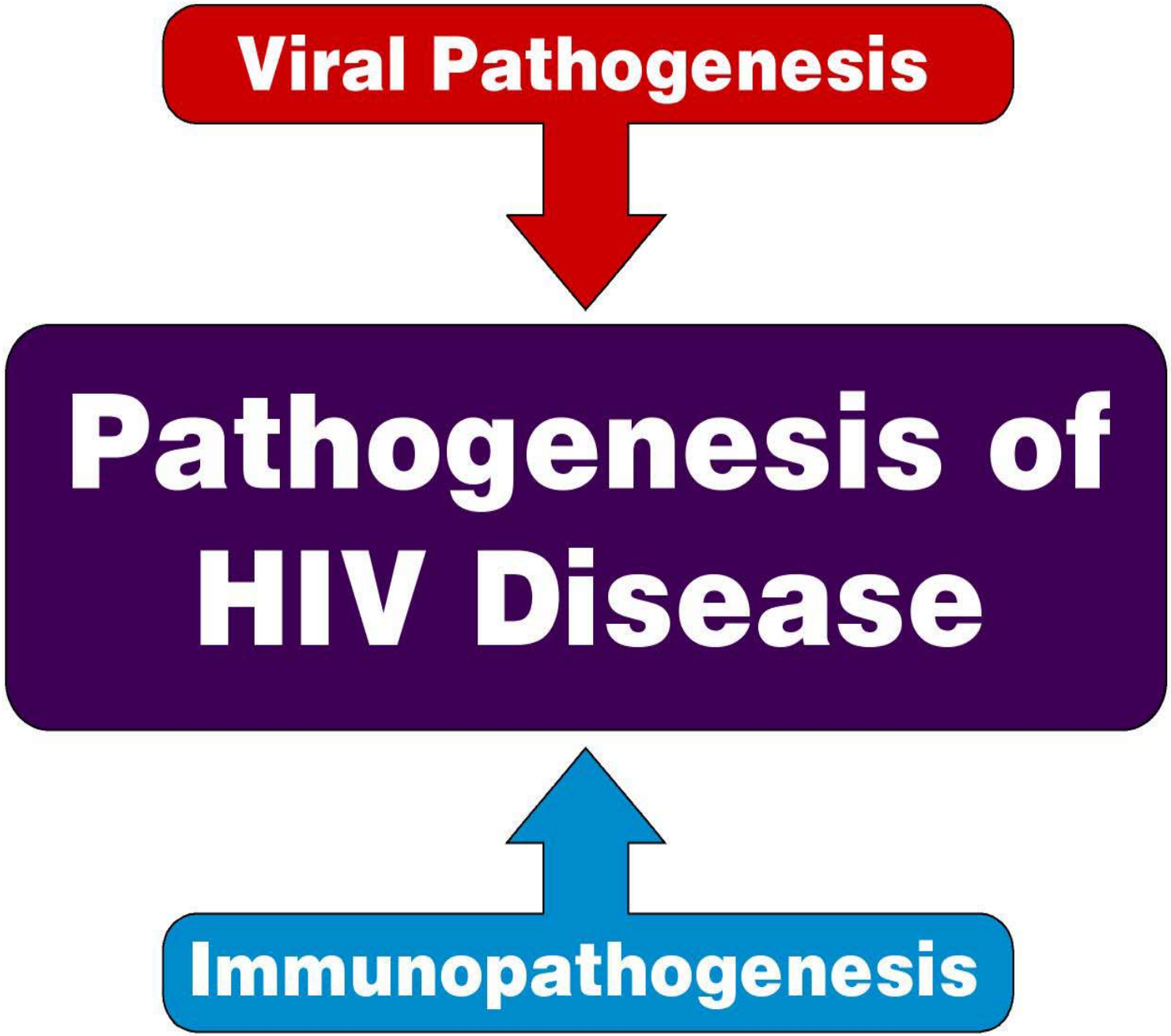
National Institutes of Health

Bethesda, Maryland, USA

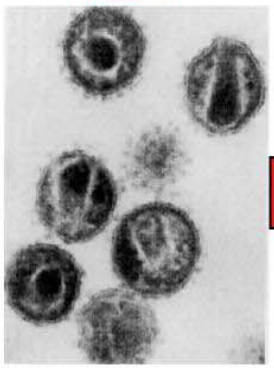
July 15, 2004


Viral Pathogenesis
Pathogenesis of HIV Disease
Immunopathogenesis

Immune Activation Drives HIV Pathogenesis

HIV Infection Leads to a State of Generalized Immune Activation

HIV

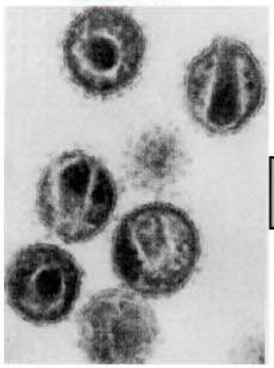


HIV Induced Immune Activation

- Increased T cell turnover (production and destruction)
- Increased activation-induced death of T cells
- A decline in the size of the $CD4^+$ T cell pool
- A state of activation-induced immunodeficiency

The Impact of ART on HIV-Associated Immune Activation

HIV

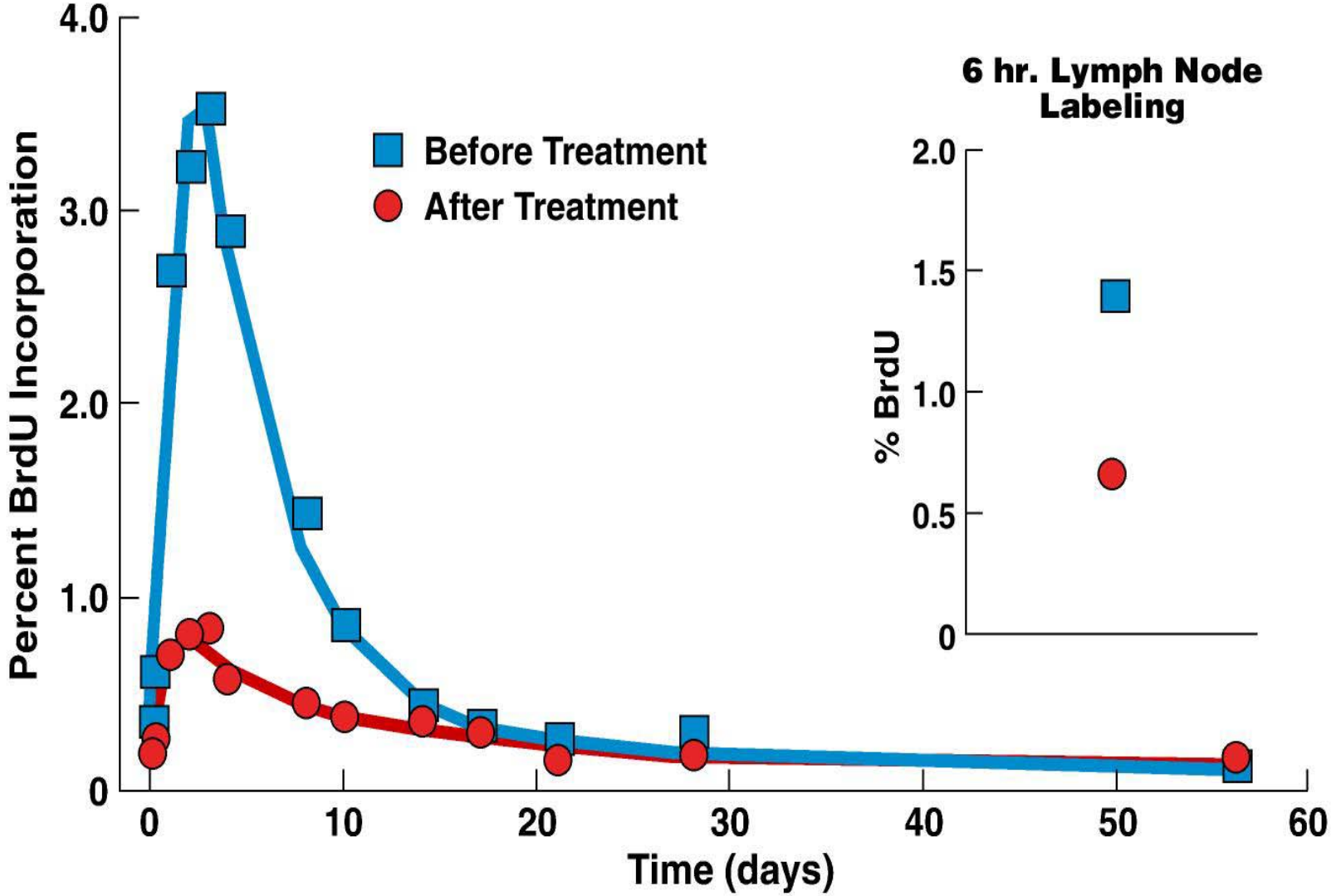


ART

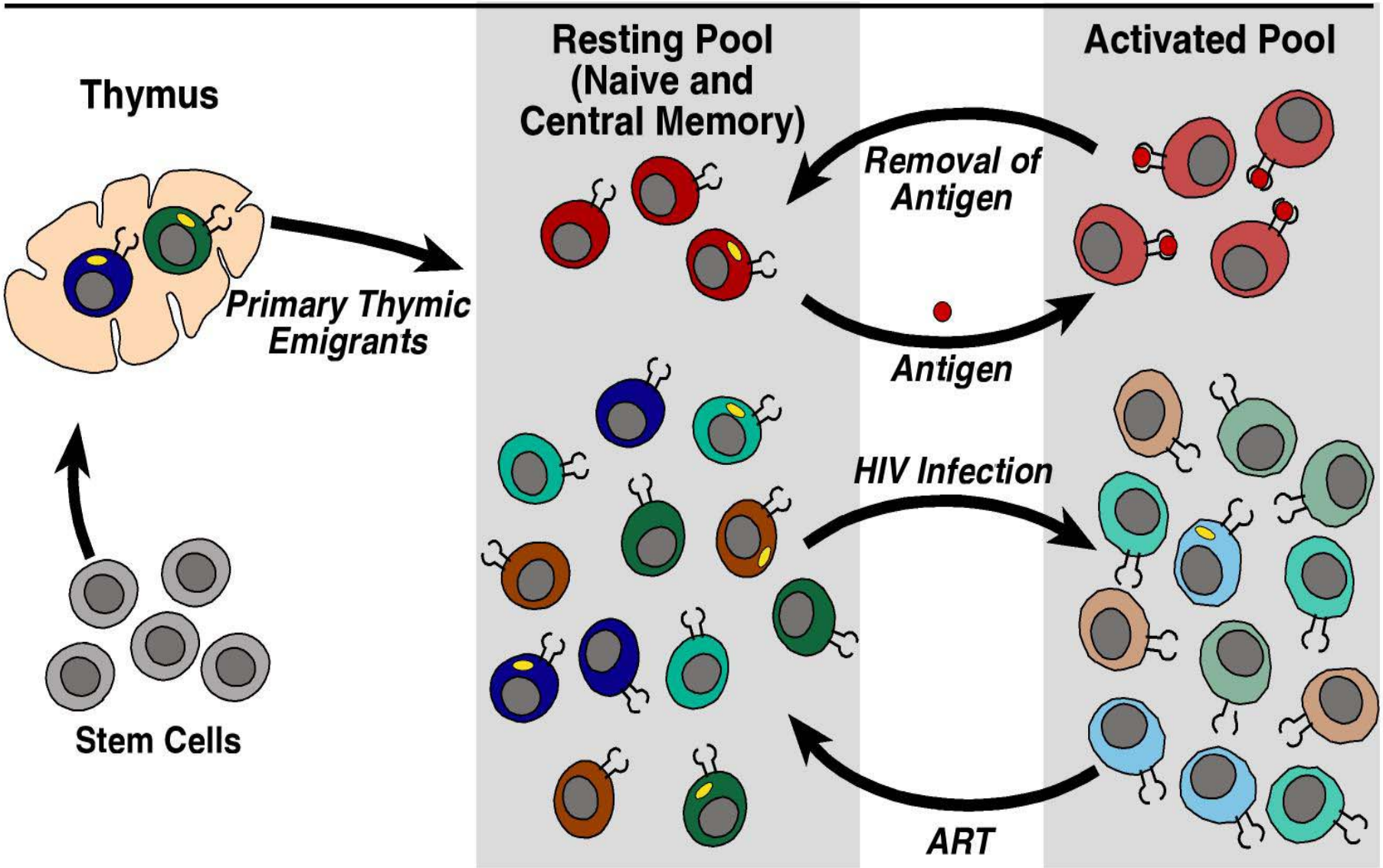
Reversal of Aberrant Immune Activation

- Decreased T cell turnover
- Increased T cell survival
- Expansion of the $CD4^+$ T cell pool
- An immediate reversal in activation-induced immunodeficiency
  - – Spontaneous resolution of opportunistic illnesses
  - – Immune reactivation syndromes

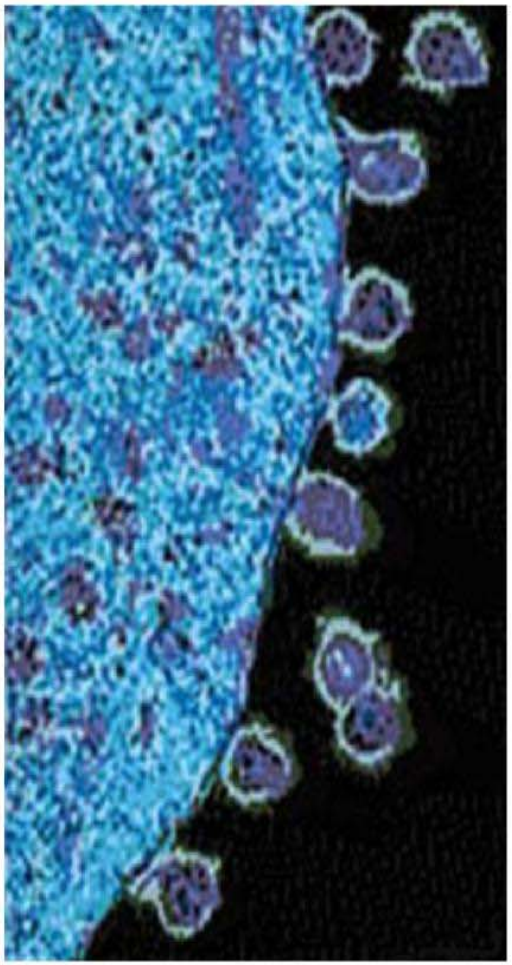
Changes in CD4+ T Cell Production Following Initiation of Antiretroviral Therapy



Impact of Antigen and HIV Infection on the $CD4^+$ T Cell Pool

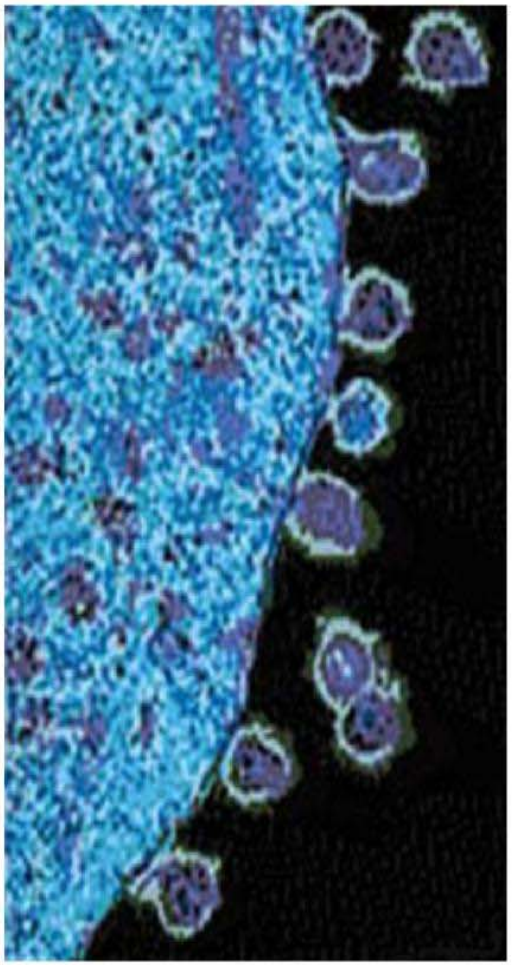


Impact of Viral Replication and Viremia on Lymphocyte Subsets in HIV-Infected Individuals



- CD4+ T Cell Reservoirs
- B Cells
- NK Cells

Impact of Viral Replication and Viremia on Lymphocyte Subsets in HIV-Infected Individuals



CD4+ T Cell Reservoirs

B Cells

NK Cells

Presence of an Inducible HIV-1 Latent Reservoir During Highly Active Antiretroviral Therapy

Tae-Wook Chun, Lieven Stuyver, Stephanie B. Mizell, Linda A. Ehler, Jo Ann M. Mican, Michael Baseler, Alun L. Lloyd, Martin A. Nowak, and Anthony S. Fauci

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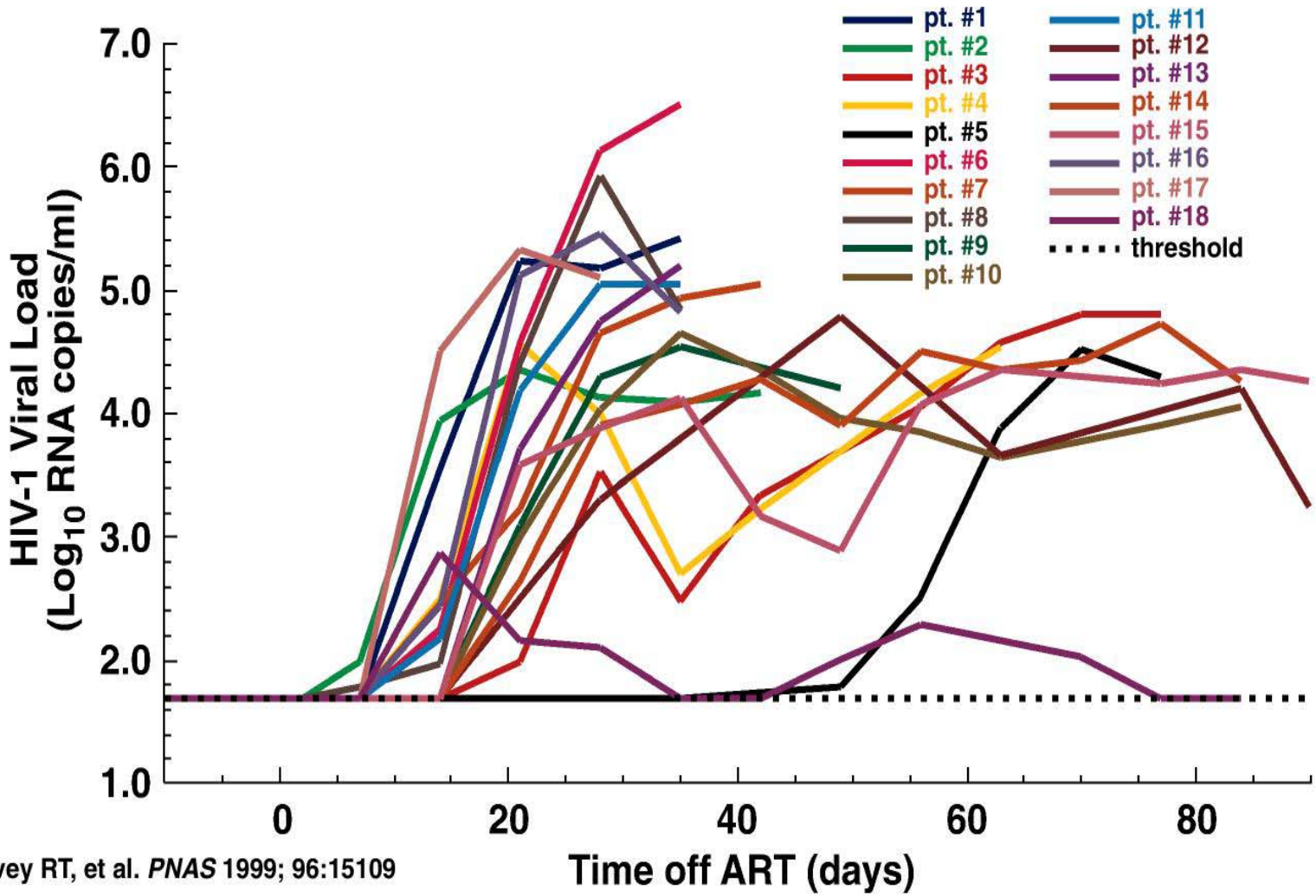
Recovery of Replication-Competent HIV Despite Prolonged Suppression of Plasma Viremia

Joseph K. Wong, Marjan Hezareh, Huldrych F. Günthard, Diane V. Havlir, Caroline C. Ignacio, Celsa A. Spina, Douglas D. Richman

Identification of a Reservoir for HIV-1 in Patients on Highly Active Antiretroviral Therapy

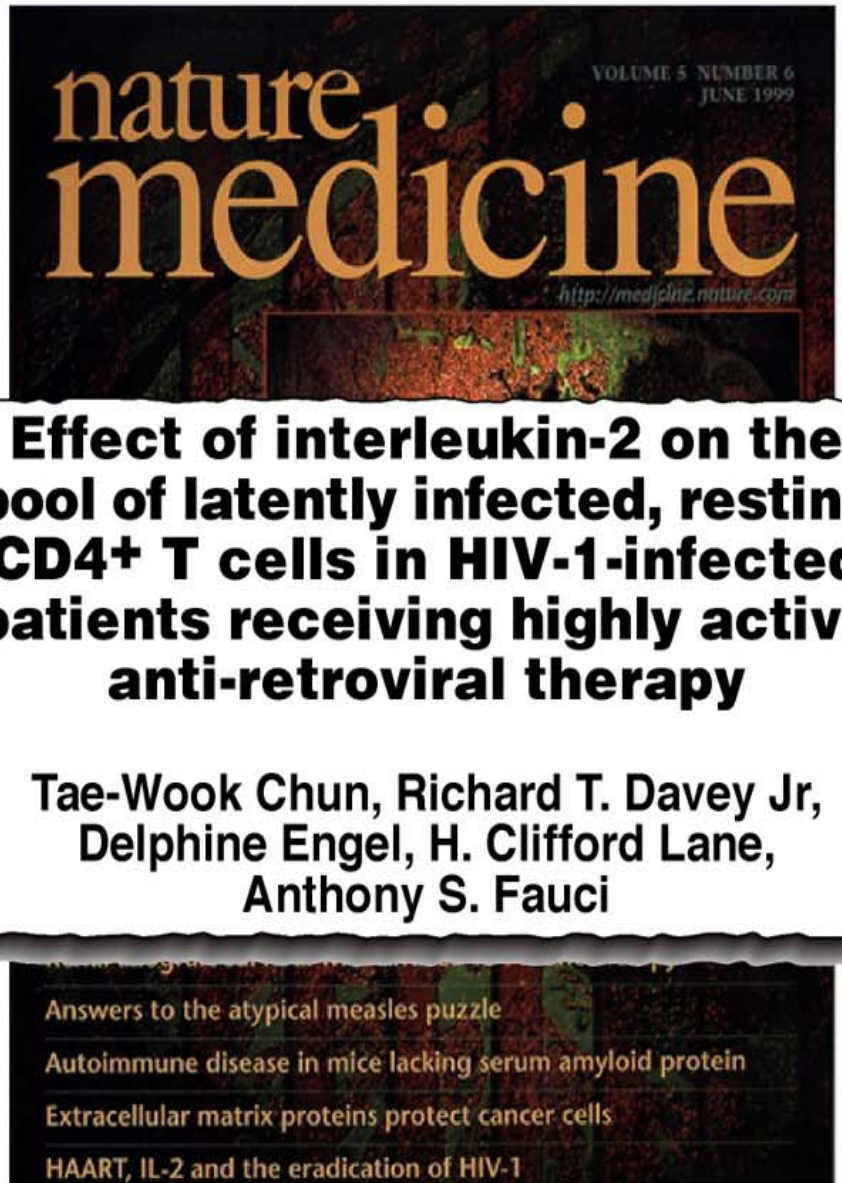
Diana Finzi, Monika Hermankova, Theodore Pierson, Lucy M. Carruth, Christopher Buck, Richard E. Chaisson, Thomas C. Quinn, Karen Chadwick, Joseph Margolick, Ronald Brookmeyer, Joel Gallant, Martin Markowitz, David D. Ho, Douglas D. Richman, Robert F. Siliciano

Viral Relapse Following Discontinuation of ART



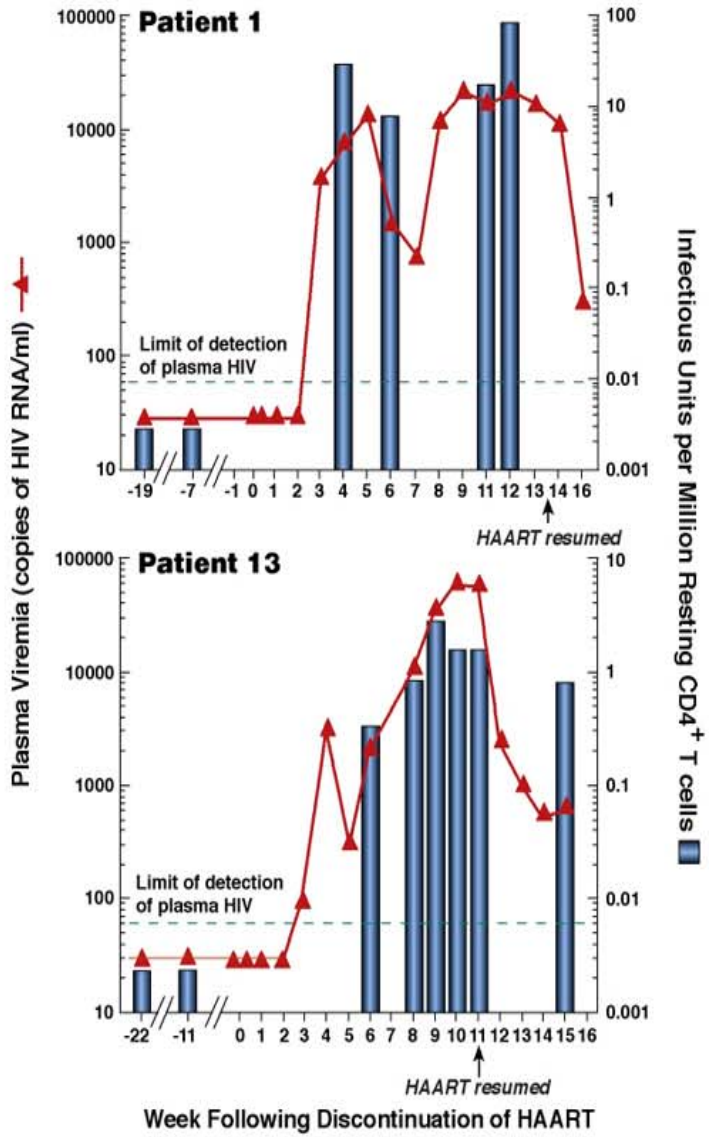
Davey RT, et al. *PNAS* 1999; 96:15109

Importance of Viremia in Replenishment of the HIV Latent Reservoir



Effect of interleukin-2 on the pool of latently infected, resting CD4+ T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy

Tae-Wook Chun, Richard T. Davey Jr, Delphine Engel, H. Clifford Lane, Anthony S. Fauci



The New England Journal of Medicine

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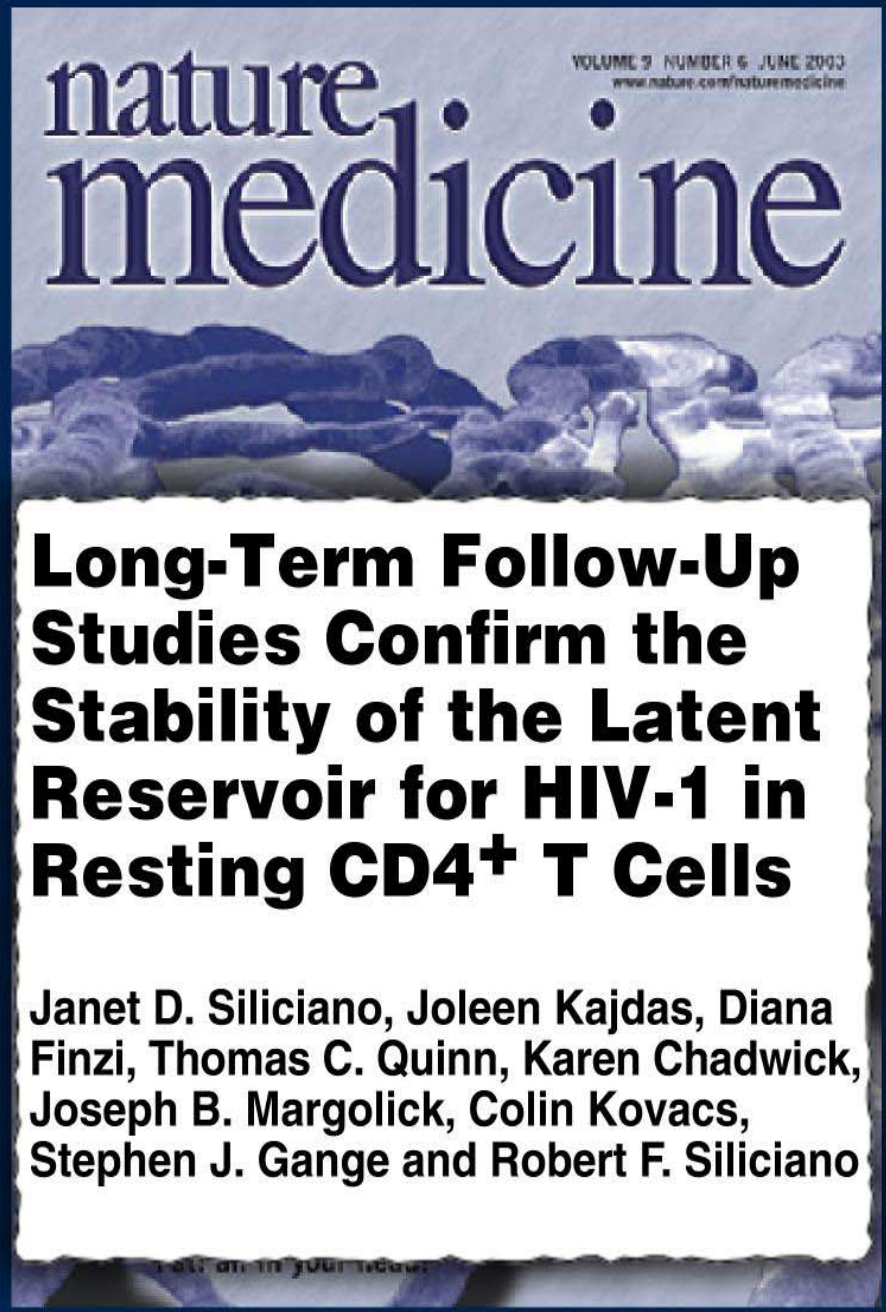
VOLUME 340 MAY 27, 1999 NUMBER 21

Quantifying Residual HIV-1 Replication in Patients Receiving Combination Antiretroviral Therapy

Linqi Zhang, Bharat Ramratnam, Klara Tenner-Racz, Yuxian He, Mika Vesanen, Sharon Lewin, Andrew Talal, Paul Racz, Alan S. Perelson, Bette T. Korber, Martin Markowitz, and David D. Ho

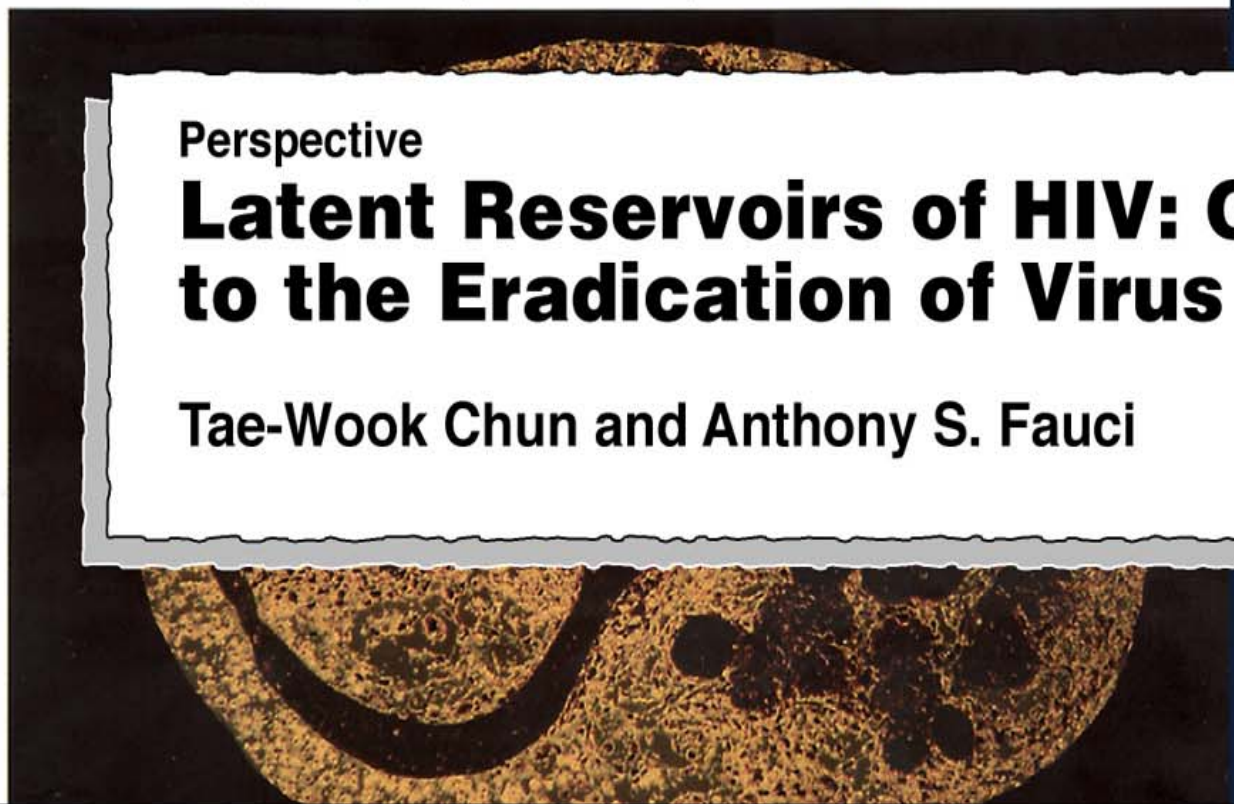


Long-Term Follow-Up Studies Confirm the Stability of the Latent Reservoir for HIV-1 in Resting CD4+ T Cells

Janet D. Siliciano, Joleen Kajdas, Diana Finzi, Thomas C. Quinn, Karen Chadwick, Joseph B. Margolick, Colin Kovacs, Stephen J. Gange and Robert F. Siliciano

PNAS
Proceedings of the National Academy of Sciences
of the United States of America

September 28, 1999 | vol. 96 | no. 20 | pp. 10945–11688 | www.pnas.org



Perspective

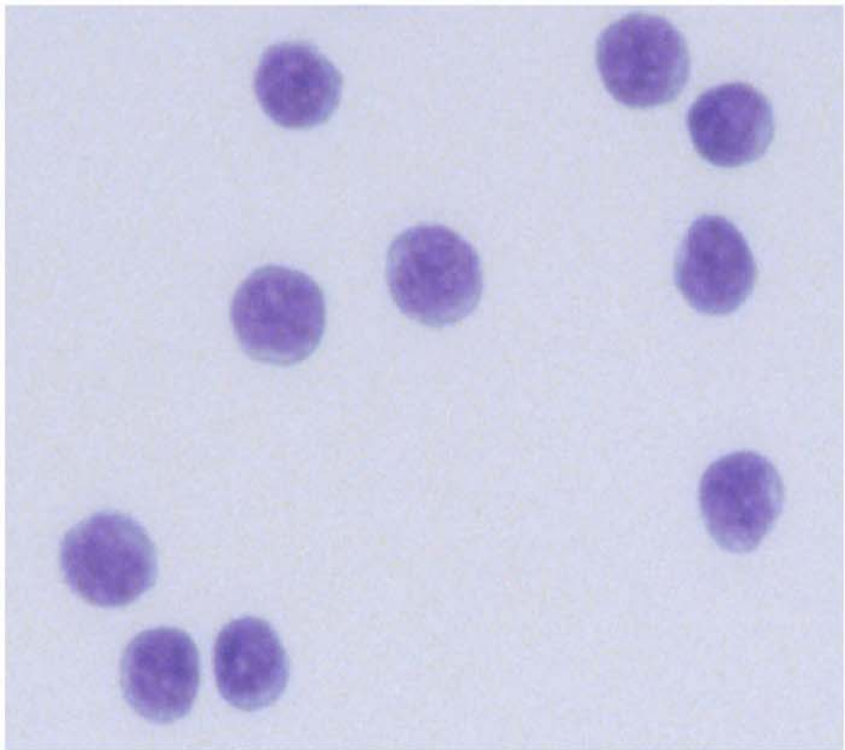
Latent Reservoirs of HIV: Obstacles to the Eradication of Virus

Tae-Wook Chun and Anthony S. Fauci

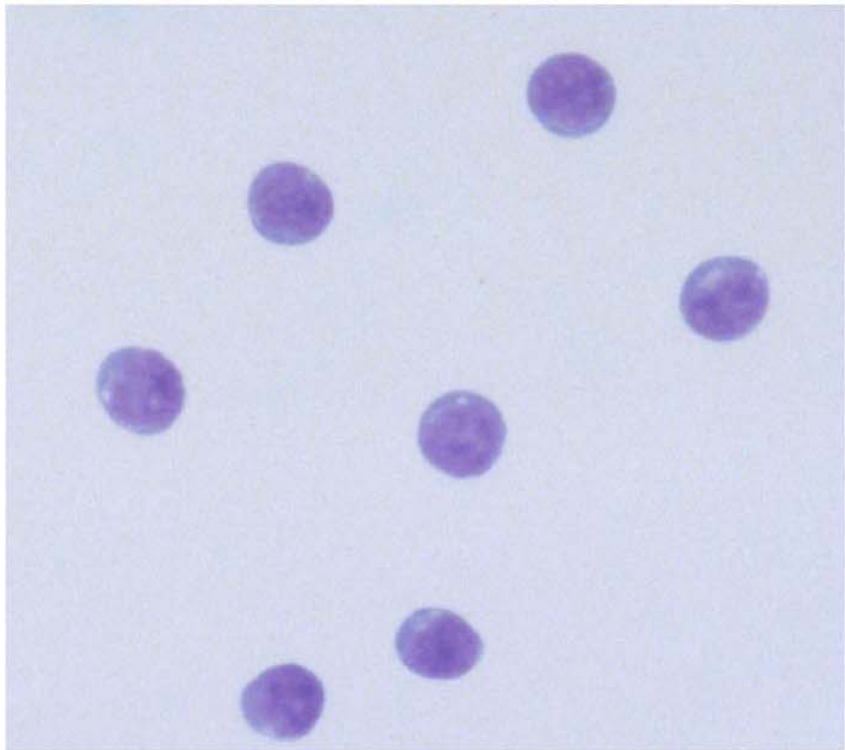
The Resting $CD4^+$ T Cell Reservoir of HIV in Viremic Versus Aviremic Individuals

Morphology of Resting CD4$^+$ T Cells

Aviremic Patient

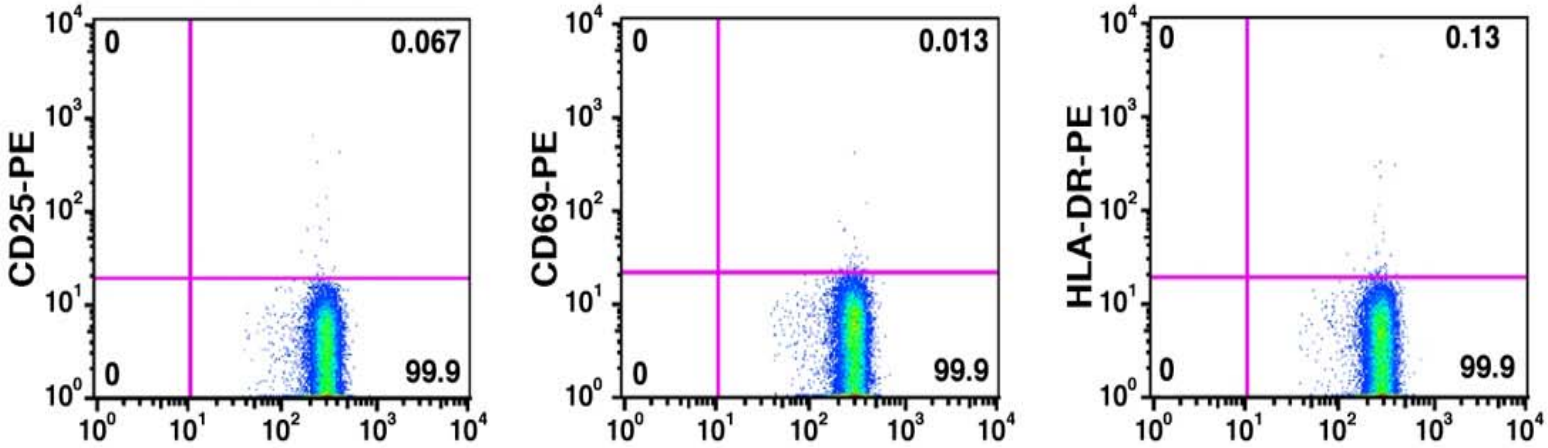


Viremic Patient

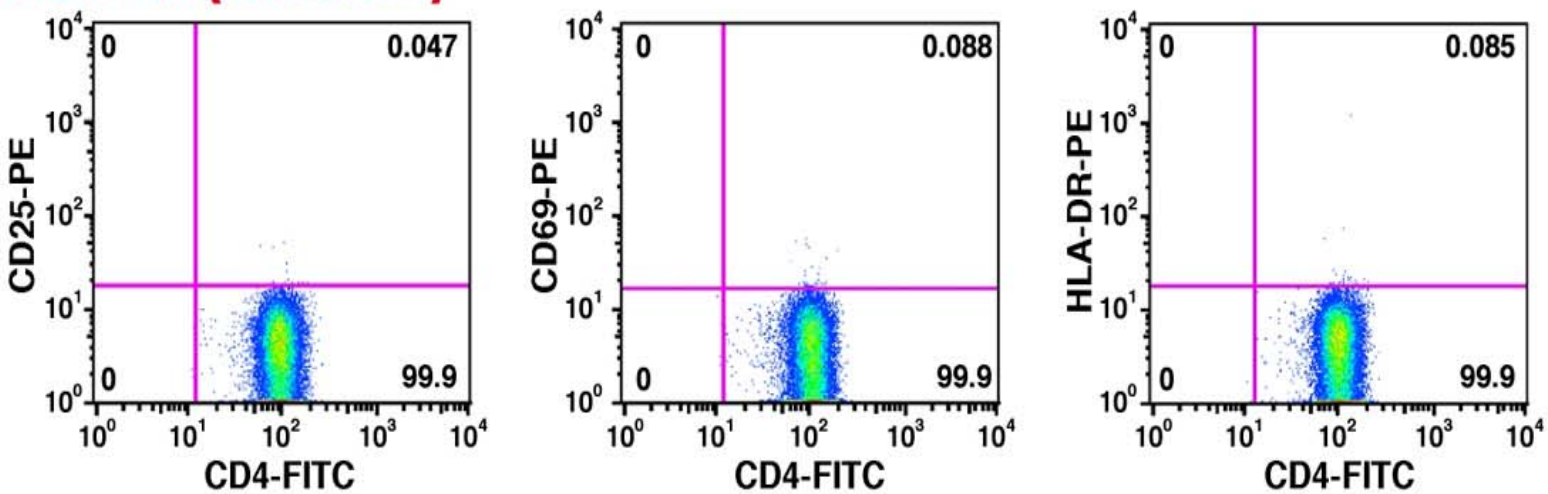


Resting CD4+ T Cell Reservoir for HIV in Viremic versus Aviremic Individuals is Phenotypically Identical

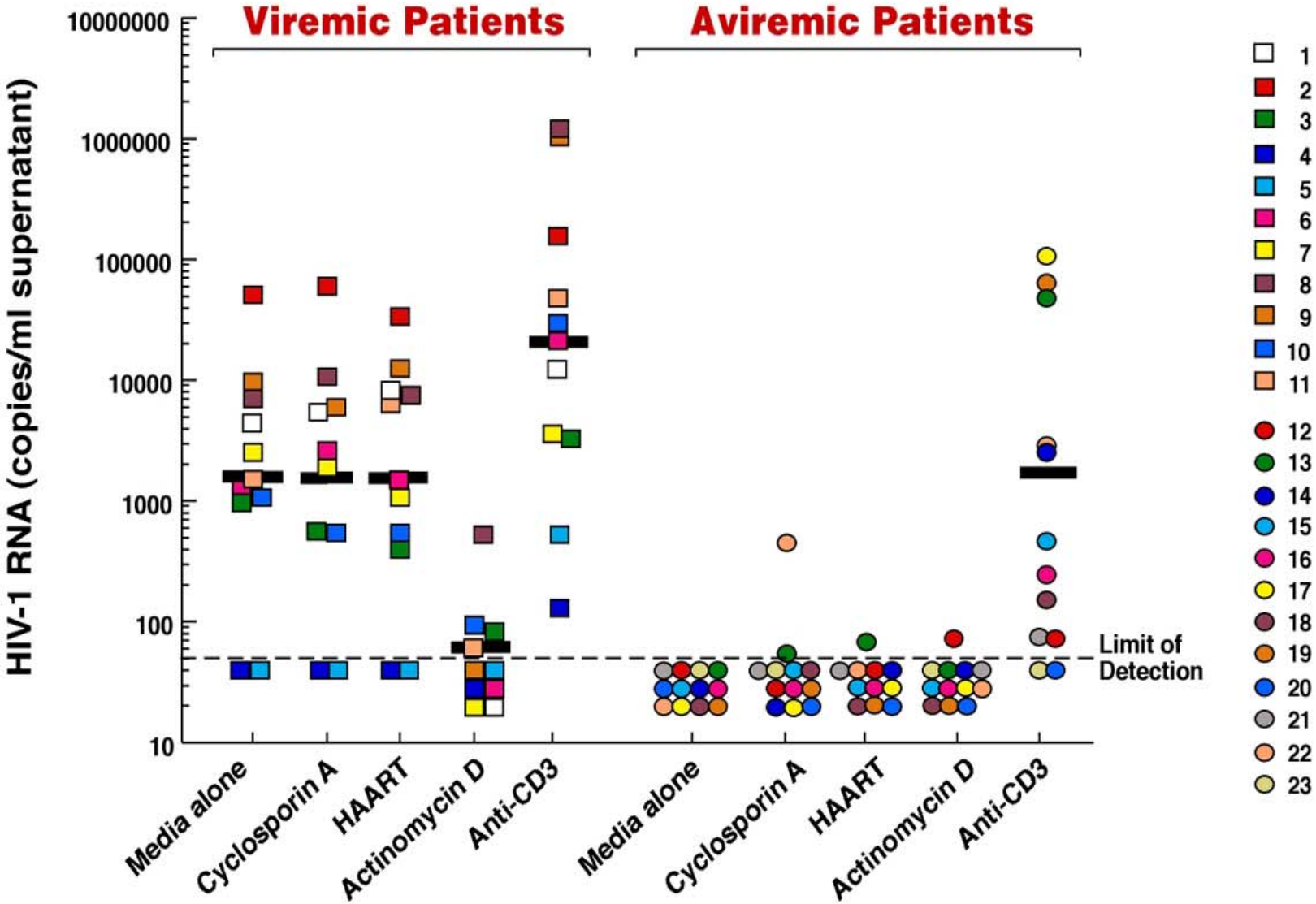
Patient 4 (Viremic)



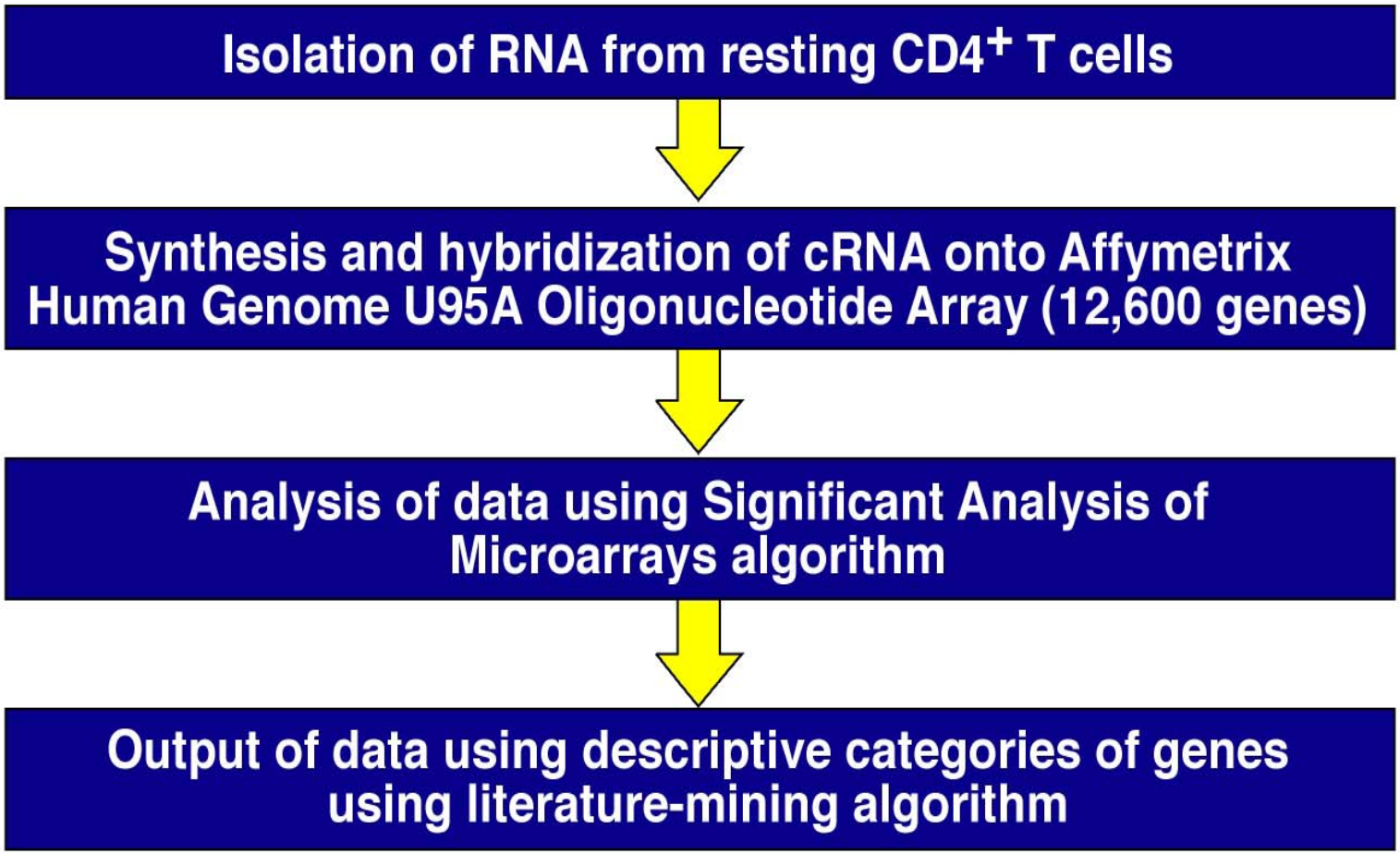
Patient 22 (Aviremic)



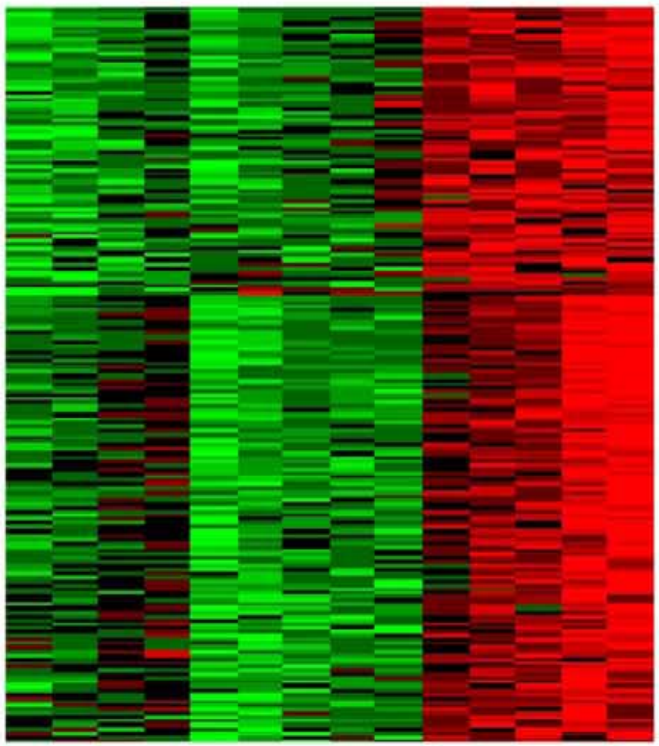
Levels of Cell-Free HIV Virions Released by Latently Infected, Resting CD4+ T Cells from Viremic and Aviremic Patients

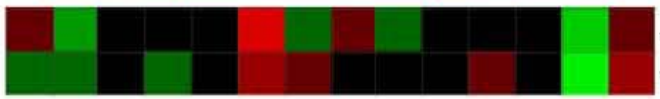


DNA Microarray Analysis of Resting CD4+ T Cells from Aviremic and Viremic HIV-Infected Patients



DNA Microarray Analysis of Resting CD4$^+$ T Cells from Aviremic and Viremic Patients and Normal Donors





– β-actin
– GAPDH

A B C D | 16 13 18 15 23 | 10 9 2 6 7

HIV-negative donors | Aviremic patient | Viremic patient

Z-Score
-2 0 2

Transcription Regulators (p=0.0004)

	Identifier	Locus	Symbol	Name
131_at	6982	TAF11		TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 28kDa
1643_g_at	9112	MTA1		metastasis associated 1
306_s_at	3150	HMGB1		high-mobility group nucleosome binding domain 1
31797_at	9519	TBPL1		TBP-like 1
31879_at	8999	FUBP3		far upstream element (FUSE) binding protein 3
32805_at	25764	MAFF		v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)
32841_at	7555	ZNF9		zinc finger protein 9 (a cellular retroviral nucleic acid binding protein)
33381_at	8202	NCOA3		nuclear receptor coactivator 3
34368_at	3066	HDAC2		histone deacetylase 2
34667_at	4799	NFX1		nuclear transcription factor, X-box binding 1
35156_at	4613	MYCN		v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
35802_at	9070	ASH2L		ash2 (absent, small, or homeotic)-like (Drosophila)
36188_at	2971	GTF3A		general transcription factor IIIA
37352_at	6672	SP100		nuclear antigen Sp100
37620_at	6883	TAF12		TAF12 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 20kDa
37929_at	4801	NFYB		nuclear transcription factor Y, beta
37974_at	10943	MSL3L1		male-specific lethal 3-like 1 (Drosophila)
38065_at	3148	HMGB2		high-mobility group box 2
38095_at	5089	PBX2		pre-B-cell leukemia transcription factor 2
38345_at	11064	CEP1		centrosomal protein 1
38426_s_at	6682	TAF11		TAF11 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 28kDa
38902_r_at1386	ATF2			activating transcription factor 2
39184_at	6929	TCEB2		transcription elongation factor B (SIII), polypeptide 2 (18kDa, elongin B)
393_s_at	861	RUNX1		runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)
39426_at	10915	TCERG1		transcription elongation regulator 1 (CA150)
39606_at	6468	SIM2		single-minded homolog 2 (Drosophila)
40791_at	5430	POLR2A		polymerase (RNA) II (DNA directed) polypeptide A, 220kDa
41360_at	9337	CNOT8		CCR4-NOT transcription complex, subunit 8
41854_at	4602	MYB		v-myb myeloblastosis viral oncogene homolog (avian)
453_at	6601	SMARCC2		SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2
891_at	7528	YY1		YY1 transcription factor

RNA Processing/Modification (p=0.0000005)

	Identifier	Locus	Symbol	Name
31950_at	26986	PABPC1		poly(A) binding protein, cytoplasmic 1
32595_at	2926	GRSF1		G-rich RNA sequence binding factor 1
32784_at	8899	PRP4		pre-mRNA processing factor 4 homolog
32799_at	22916	NCBP2		nuclear cap binding protein subunit 2, 20kD
33943_at	3187	HNRPH1		heterogeneous nuclear ribonucleoprotein H1
34647_at	1655	DDX5		RNA helicase, 68kD
34793_at	10291	SF3A1		splicing factor 3a, subunit 1, 120kD
35136_at	55916	P15-2		tyrosyl-tRNA protein P15-2
35921_at	11157	LSM6		Sm protein F
36654_s_at3181	HNRPA2B1			heterogeneous nuclear ribonucleoprotein A2/B1
36813_at	7884	SLBP		stem-loop (histone) binding protein
36966_s_at11340	DIP2			Dpa-interacting protein 2
38040_at	10285	SPF30		splicing factor 30
38527_at	4961	NONO		non-POU domain containing, octamer-binding
38828_s_at9570	KHSRP			KH-type splicing regulatory protein
39050_at	8106	PABPN1		poly(A) binding protein, nuclear 1
39415_at	3190	HNRPK		heterogeneous nuclear ribonucleoprotein K
39702_at	10236	HNRPR		heterogeneous nuclear ribonucleoprotein R
39894_g_at	27292	HSA9761		putative dimethyladenosine transferase
40453_s_at	6430	SFRS5		splicing factor, arginine/serine-rich 5
41131_f_at	3188	HNRPH2		heterogeneous nuclear ribonucleoprotein H2
41133_at	10146	G3BP		Ras-GTPase-activating protein

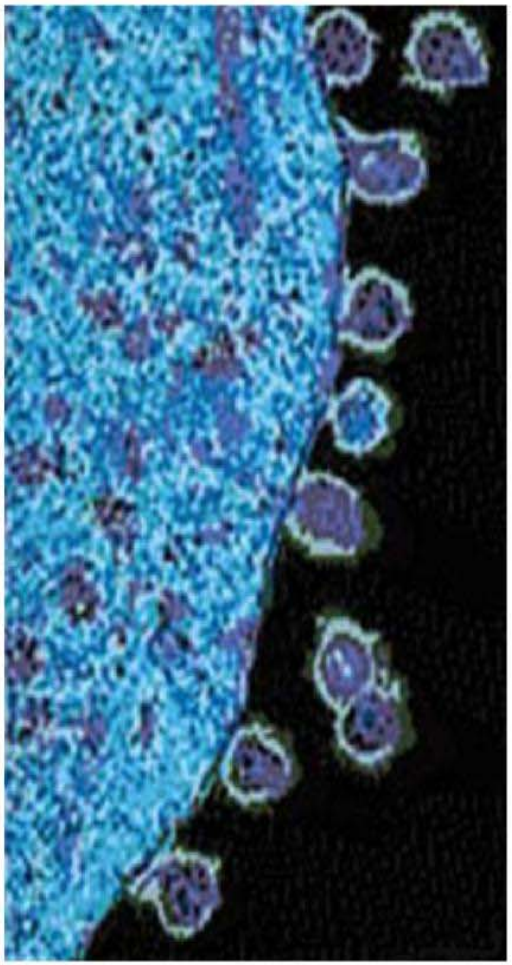
Protein Trafficking/Vesicle Transport (p=0.00002)

	Identifier	Locus	Symbol	Name
32179_s_at	8773	SNAP23		synaptosomal-associated protein, 23kD
35422_at	8996	SEC13L1		SEC13-like 1
33893_at	25256	RAI10		vesicle transport-related protein
34199_at	10802	SEC24A		SEC24 related gene family, member A
34370_at	372	ARCN1		archain 1
34796_at	25471	TRAM		translocating chain-associating membrane protein
34879_at	8813	DPM1		dolichyl-phosphate mannosyltransferase polypeptide 1
37356_r_at9815	P115			vesicle docking protein
37562_at	5869	RAB5B		RAB5B, member RAS oncogene family
37727_i_at	5955	RCN2		reticulocalbin 2, EF-hand calcium binding domain
37795_at	433	ASGR2		asialoglycoprotein receptor 2
37969_at	25163	GGA3		golgi associated ARF binding protein 3
38074_at	1175	AP3S1		adaptor-related protein complex 3, sigma 1 subunit
38505_at	10565	BIG1		brefeldin A-inhibited guanine nucleotide-exchange protein 1
38619_at	8570	GOSR2		golgi SNAP receptor complex member 2
38774_at	8417	STX7		syntaxin 7
38924_s_at10006	SH3GSP1			spectrin SH3 domain-binding protein 1
39706_at	8895	CPNE3		copine III
40147_at	10493	VAT1		vesicle amine transport protein 1
41462_at	8543	SNX2		sorting nexin 2

Conclusions

- There is a fundamental difference in the resting $CD4^+$ T cell reservoir in viremic versus aviremic patients. In the former, true latency likely does not exist as cells are continually poised to express virus. In the latter, much greater stability exists and this may represent a truly latent reservoir of virus.
- Active viral replication, as manifested by detectable plasma viremia, has a significant impact on the physiologic state of resting $CD4^+$ T cells in infected viremic patients, and in turn, allows release of cell-free HIV-1 without exogenous activation stimuli.

Impact of Viral Replication and Viremia on Lymphocyte Subsets in HIV-Infected Individuals



CD4+ T Cell Reservoirs

B Cells

NK Cells

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VOLUME 309 August 25, 1983 NUMBER 8

Abnormalities of B-Cell Activation and Immunoregulation in Patients with the Acquired Immunodeficiency Syndrome

H.C. Lane, H. Masur, L.C. Edgar, G. Whalen, A.H. Rook, and A.S. Fauci

PNAS
Proceedings of the National Academy of Sciences
of the United States of America

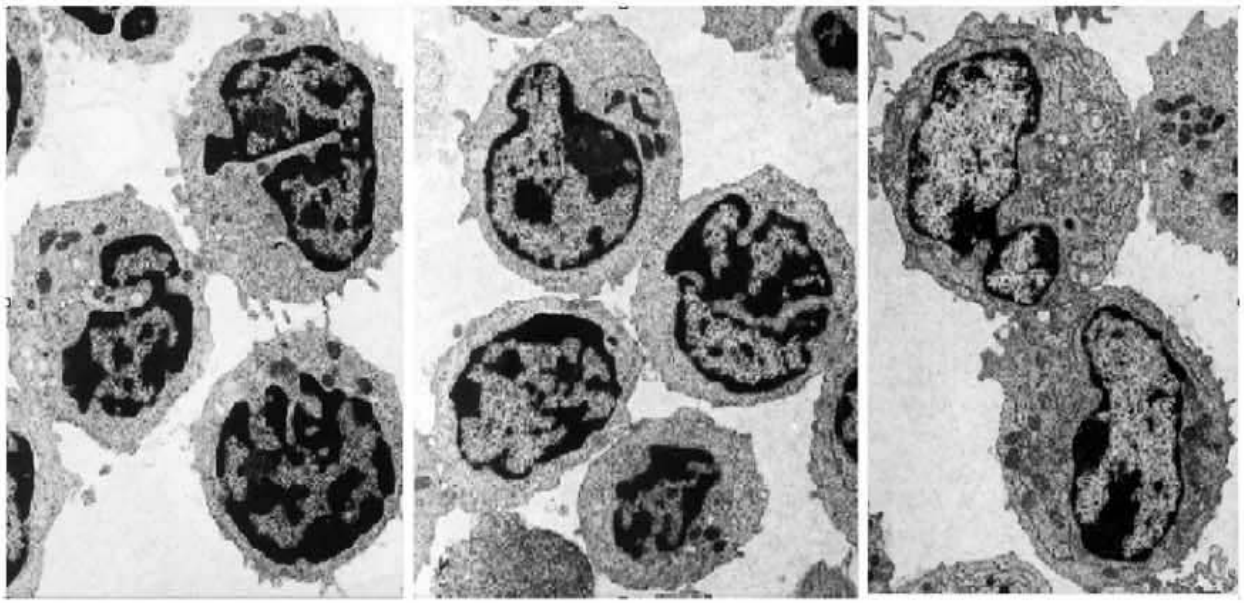
August 28, 2001 | vol 98 | no. 18

HIV-1 Induces Phenotypic and Functional Perturbations of B Cells in Chronically Infected Individuals

Susan Moir, Angela Malaspina, Kisani M. Ogwaro, Eileen T. Donoghue, Claire W. Hallahan, Linda A. Ehler, Shuying Liu, Joseph Adelsberger, Réjean Lapointe, Patrick Hwu, Michael Baseler, Jan M. Orenstein, Tae-Wook Chun, Jo Ann M. Mican, and Anthony S. Fauci

Previous Findings Indicate That HIV-Mediated Hyper-Activation Induces Terminal Differentiation of B Cells

- Loss of CD21 expression
- Reduced proliferation
- Increased immunoglobulin secretion
- Changes in morphology

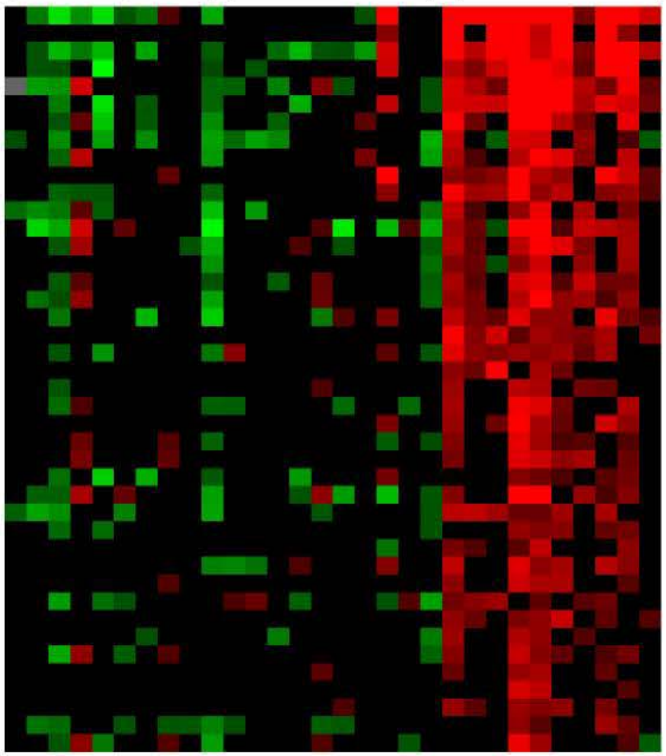


HIV-negative HIV-viremic CD21+ HIV-viremic CD21−

Determination of B Cell Gene Expression in Viremic versus Aviremic HIV-Infected versus HIV-Negative Individuals

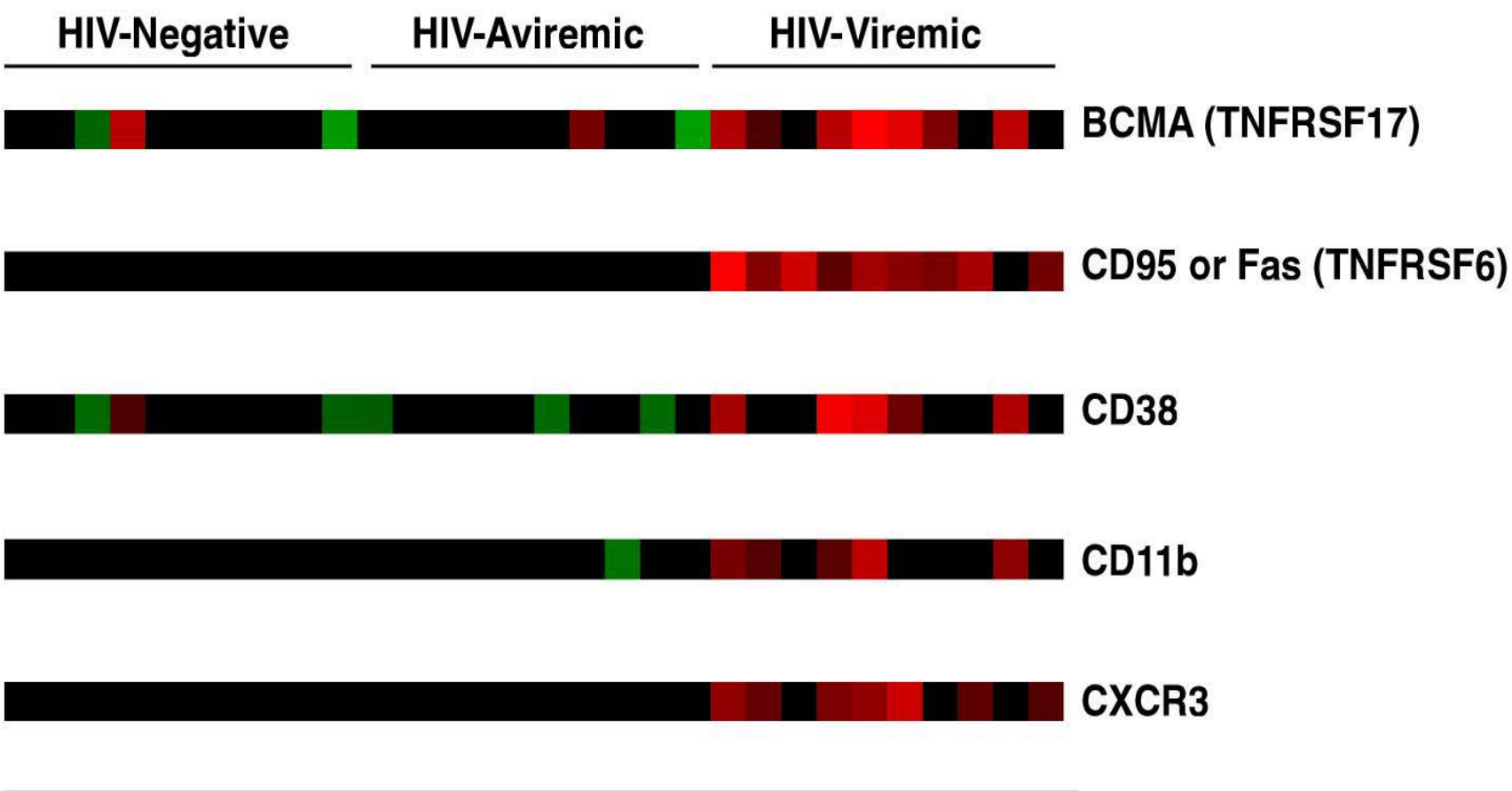
DNA Microarray Analysis: Genes Upregulated in B Cells of HIV-Viremic Patients

- Major categories of genes upregulated:
  - Interferon-stimulated genes
  - Genes associated with B cell terminal differentiation

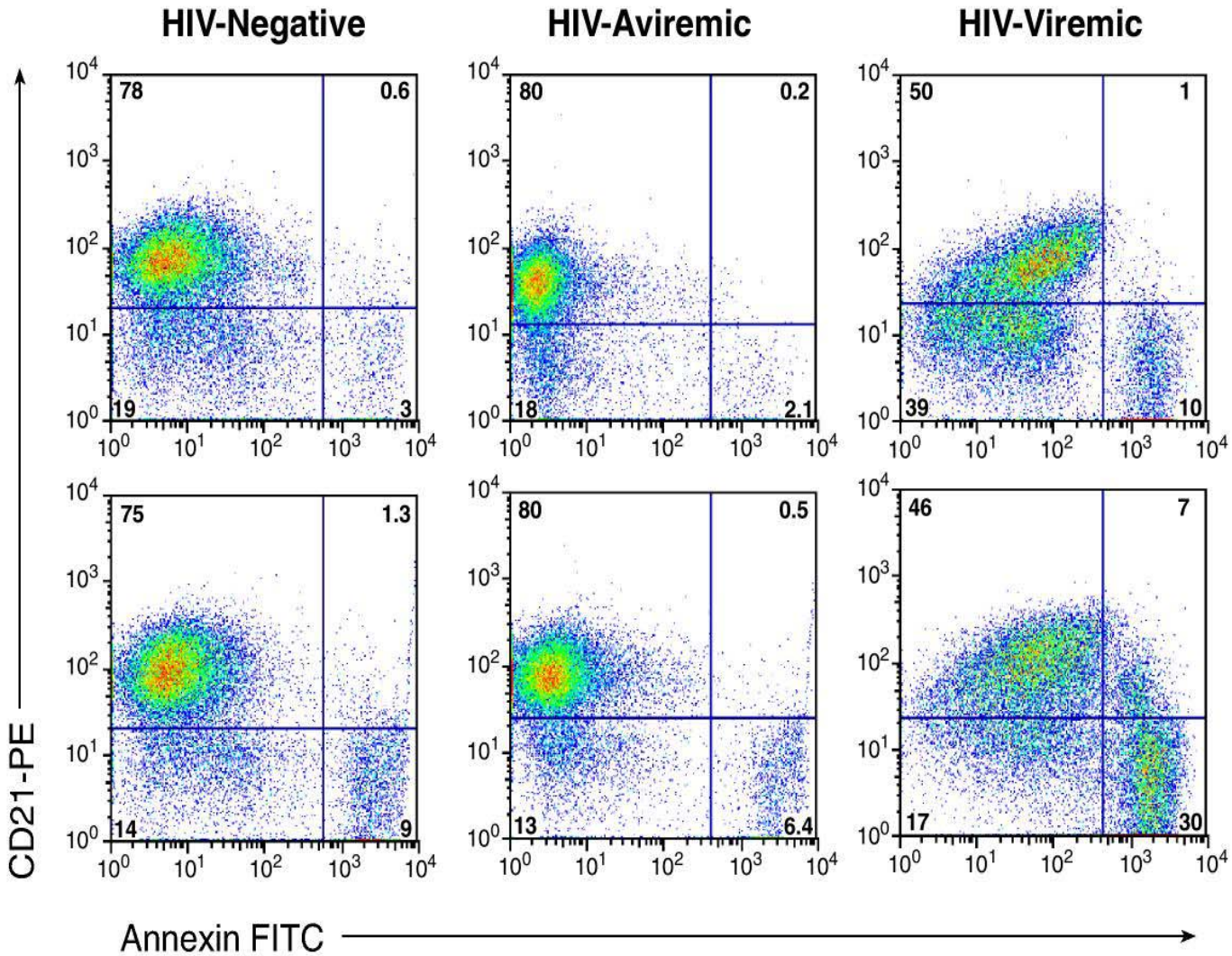


Accession no.	Gene description	Category
NM_006820	histocompatibility 28	
NM_005532	interferon, alpha-inducible protein 27 (IFI27)	ISG
NM_006417	interferon-induced protein 44 (IFI44)	ISG
NM_005101	interferon-stimulated protein, 15 kDa (ISG15)	ISG
NM_003490	synapsin III (SYN3), transcript variant IIIa	TD
NM_002462	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78	ISG
XM_037670	immunoglobulin lambda-like mRNA (F lambda 1 clone)	TD
NM_001915	cytochrome b-561 (CYB561)	TD
NM_001192	tumor necrosis factor receptor superfamily, member 17 (TNFRSF17) **BCMA**	TD
NM_001549	interferon-induced protein with tetratricopeptide repeats 4 (IFIT4)	ISG
	interferon-inducible protein 9-27	ISG
NM_004646	congenital, Finnish type (nephrin) (NPHS1)	
	DNA sequence of the human immunoglobulin D segment	TD
NM_032470	tenascin XB (TNXB), transcript variant XB-S	TD
NM_130786	alpha-1-B glycoprotein (A1BG)	TD
NM_004733	acetyl-Coenzyme A transporter (ACATN)	
NM_000381	midline 1 (Opitz/BBB syndrome) (MID1), transcript variant 1	
NM_002534	2',5'-oligoadenylate synthetase 1 (40-46 kD) (OAS1), transcript variant E16,	ISG
NM_000043	tumor necrosis factor receptor superfamily, member 6 (TNFRSF6; **CD95)**	ISG
NM_006332	interferon, gamma-inducible protein 30 (IFI30)	ISG
XM_010879	retinol dehydrogenase mRNA	
NM_001825	creatine kinase, mitochondrial 2 (sarcomeric) (CKMT2)	
NM_001775	CD38 antigen (p45) **(CD38)**	TD
NM_000319	peroxisome receptor 1 (PXR1)	
NM_006010	arginine-rich, mutated in early stage tumors (ARMET)	TD
NM_004925	aquaporin 3 (AQP3)	TD
NM_007315	signal transducer and activator of transcription 1, 91kD (STAT1)	ISG
NM_005512	glycoprotein A repetitions predominant (GARP)	
NM_005620	S100 calcium binding protein A11 (calgizzarin) (S100A11)	TD
NM_015907	leucine aminopeptidase 3 (LAP3)	
NM_000632	**CD11b**	TD
NM_022873	interferon, alpha-inducible protein (clone IFI-6-16) (G1P3), transcript variant 3	ISG
NM_001034	ribonucleotide reductase M2 polypeptide	
NM_004848	basement membrane-induced gene (ICB-1)	TD
NM_001504	G protein-coupled receptor 9 (GPR9) **CXCR3**	TD
NM_004039	annexin A2 (ANXA2); receptor for tenascin C	ISG
NM_007107	signal sequence receptor, gamma (translocon-associated protein gamma)	TD
NM_004032	D-aspartate oxidase	
NM_016594	FK506 binding protein 11	TD
NM_005533	Similar to interferon-induced protein 35	ISG
NM_002201	interferon stimulated gene (20kD) (ISG20)	ISG
NM_016459	proapoptotic caspase adaptor protein (FLJ32987)	TD

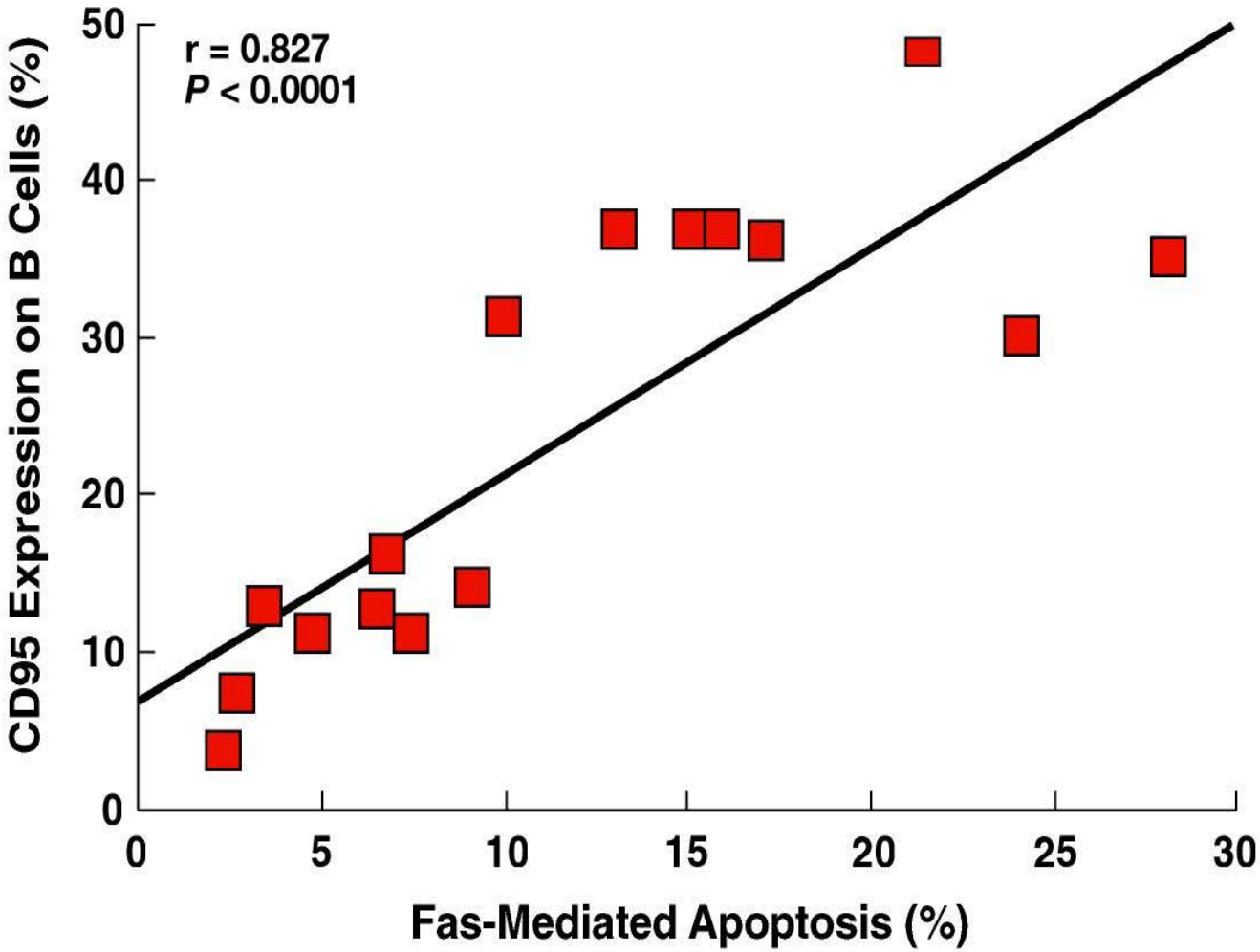
Microarray Sub-analysis: Genes of Cell Surface Receptors Associated with Interferon Induction and B Cell Terminal Differentiation



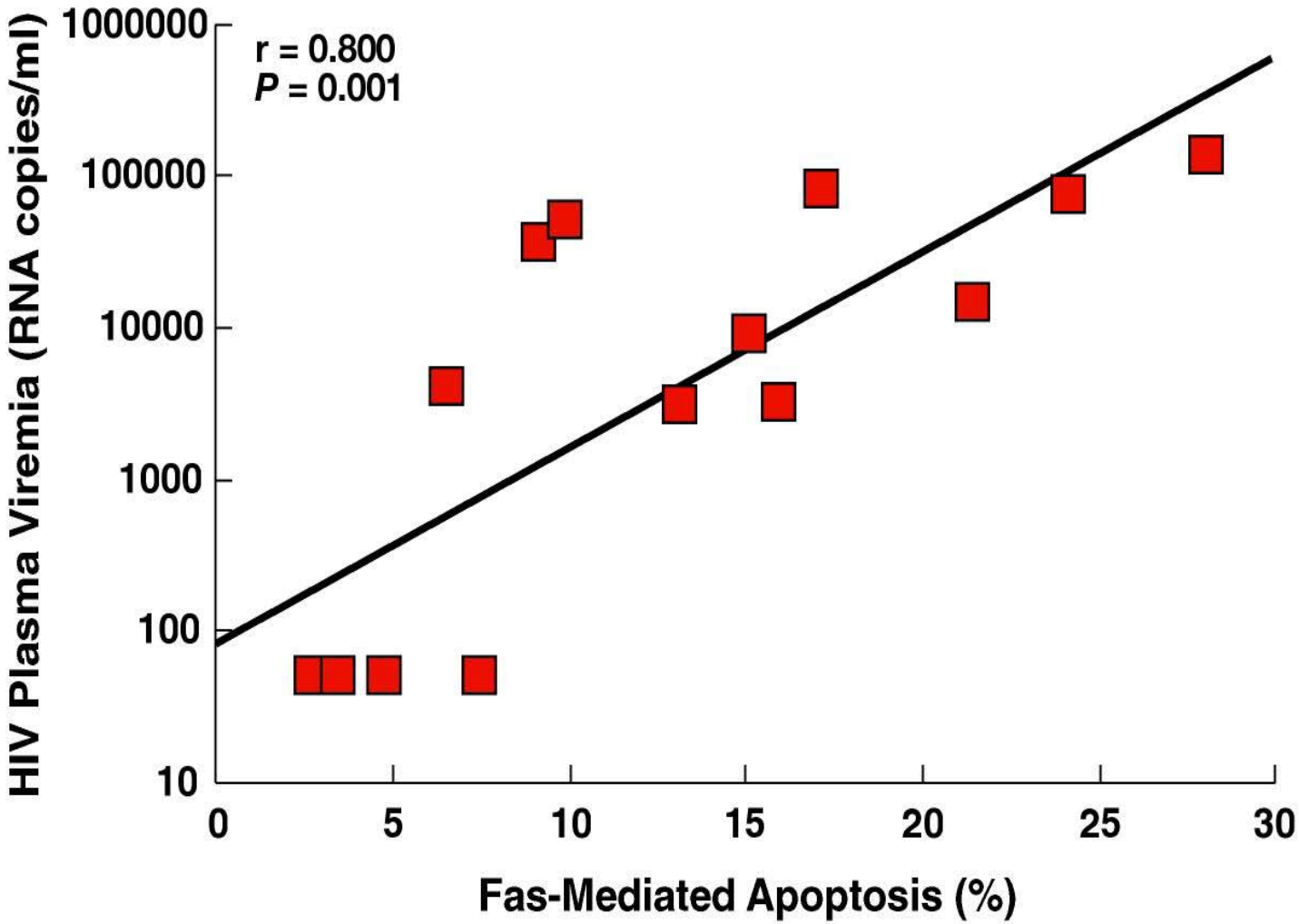
Increased Susceptibility of B Cells of HIV-Viremic Patients to Fas-Mediated Apoptosis



Direct Correlation Between Level of Fas Expression on B Cells and Susceptibility of B Cells to Fas-Mediated Apoptosis



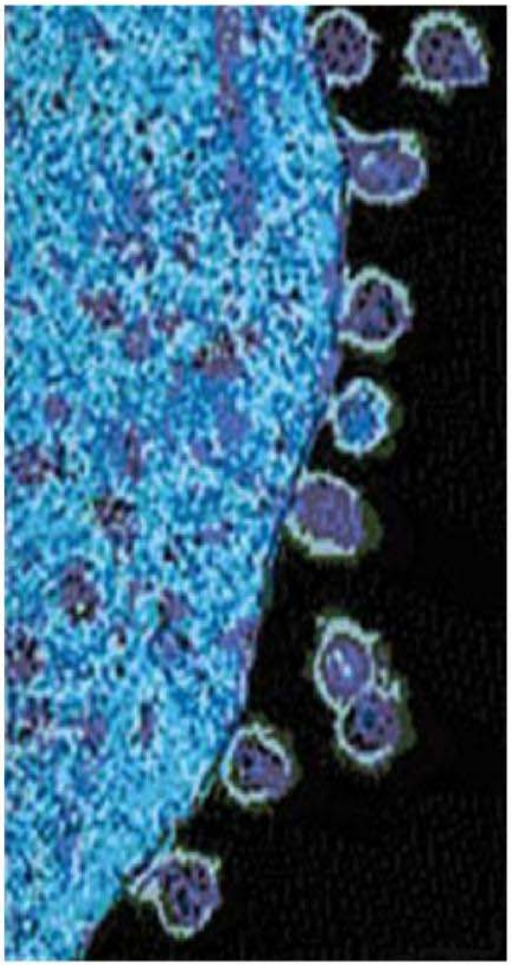
Direct Correlation Between HIV Plasma Viremia and Susceptibility of B Cells to Fas-Mediated Apoptosis



Summary

- A large number (~42) of B cell genes are upregulated in HIV-viremic vs HIV-aviremic and HIV-negative individuals; 75% are interferon-stimulated or associated with B cell terminal differentiation.
- Changes in expression of TNFSF receptors such as CD95 (Fas) are associated with increased B cell death by apoptosis.

Impact of Viral Replication and Viremia on Lymphocyte Subsets in HIV-Infected Individuals

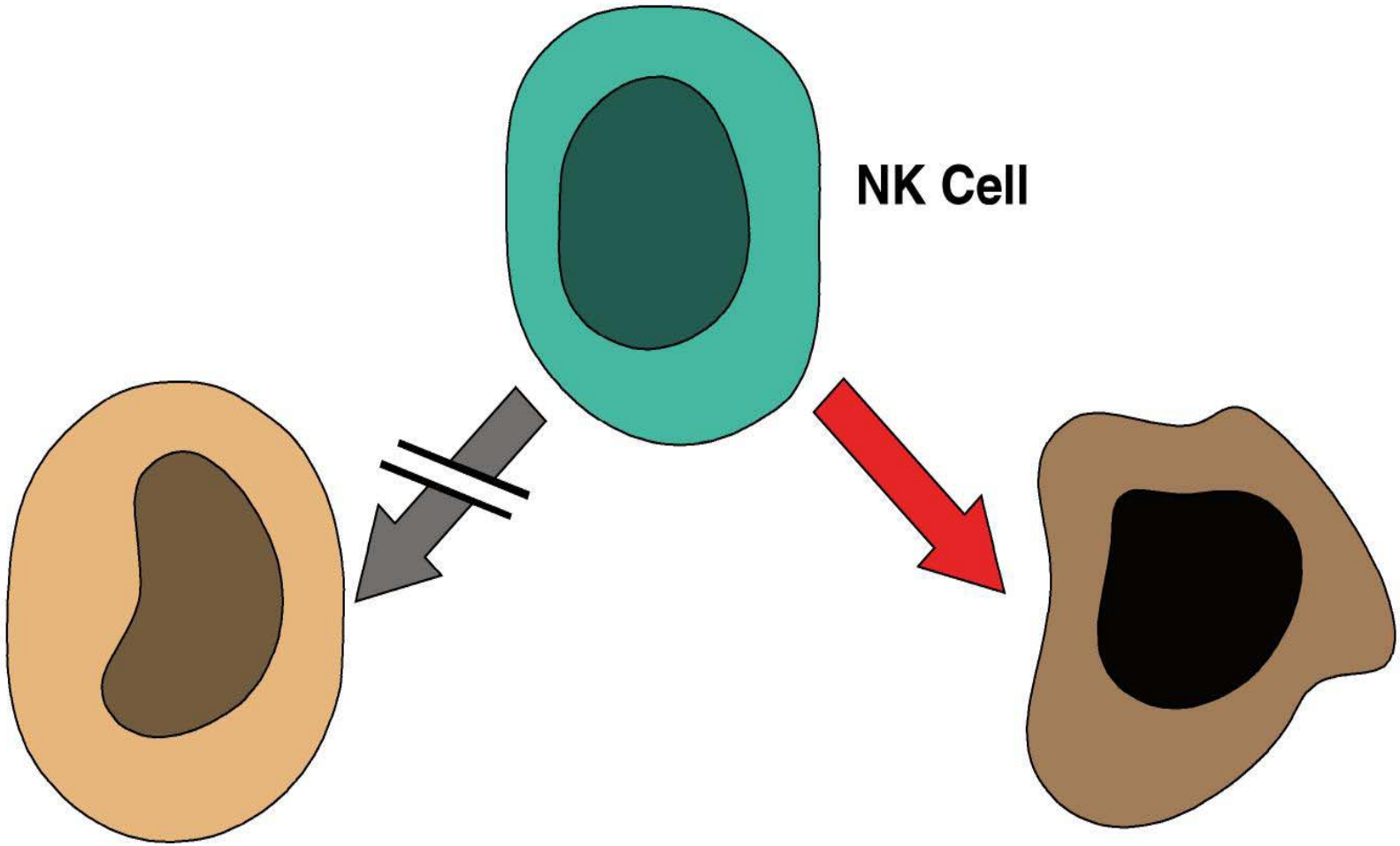


CD4+ T Cell Reservoirs

B Cells

NK Cells

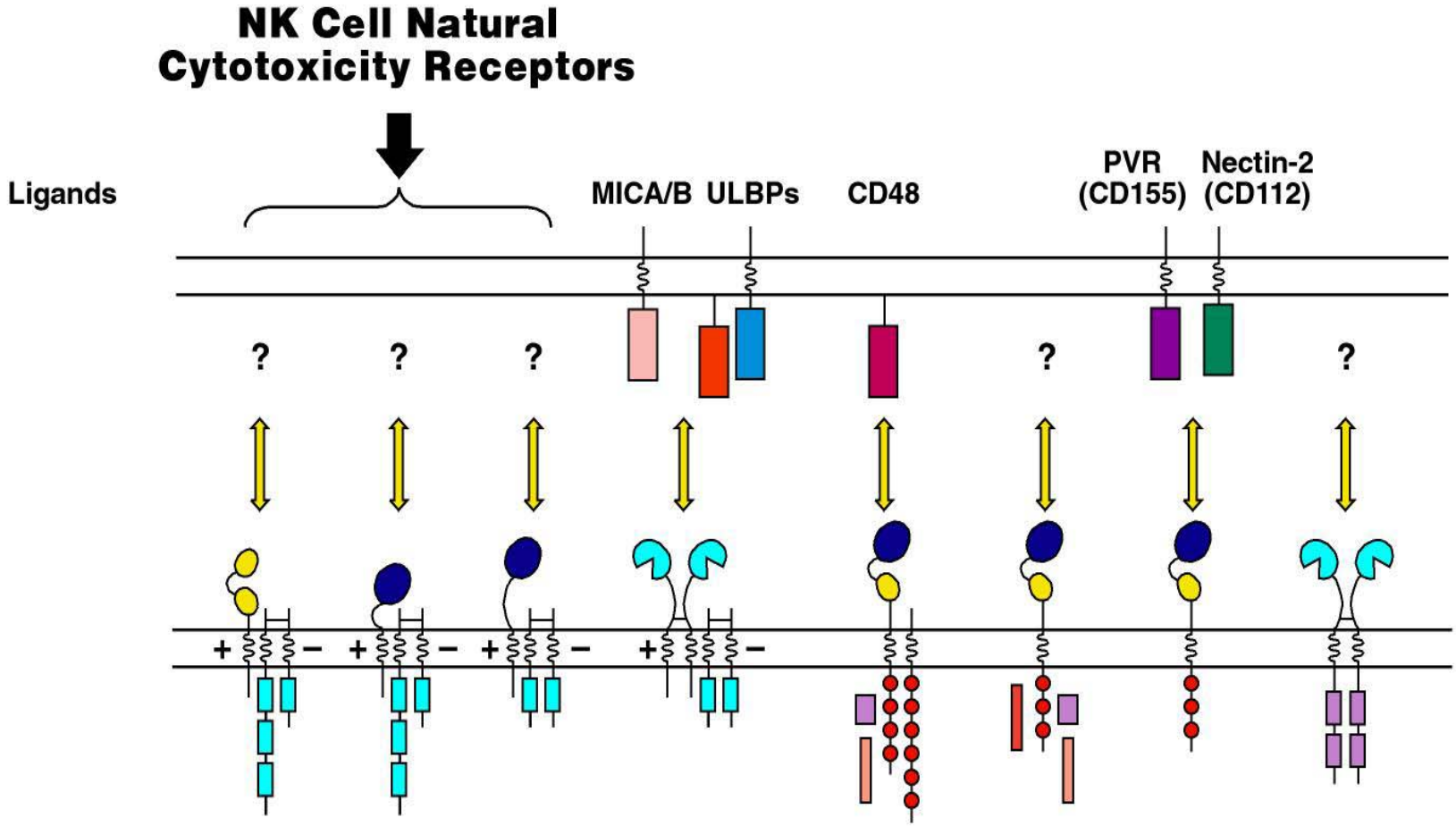
Function of NK Cells



2 Major Types of NK Cell Receptors

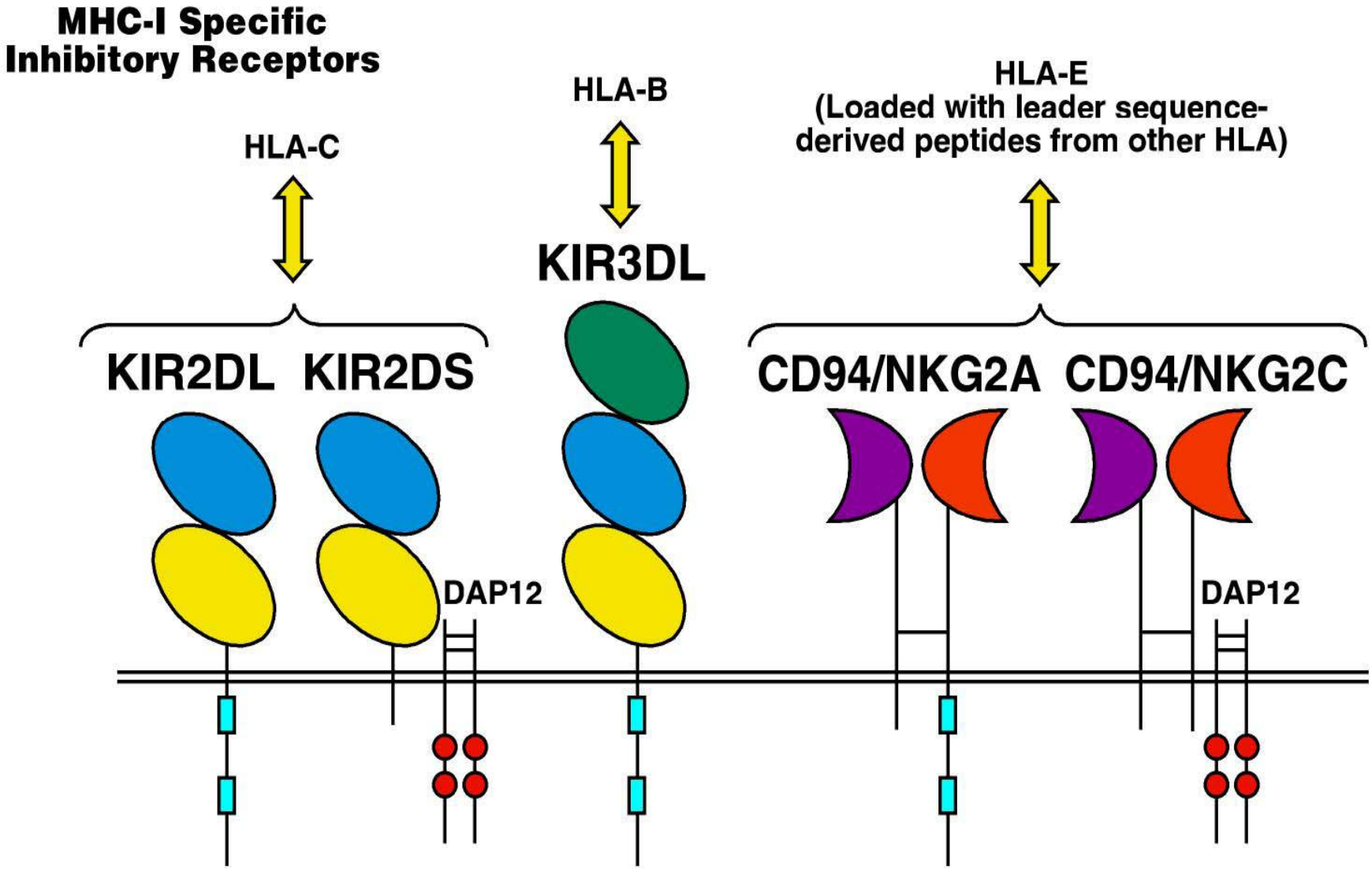
- Activating
- Inhibitory (Dominant)

NK Cell Activating Receptors

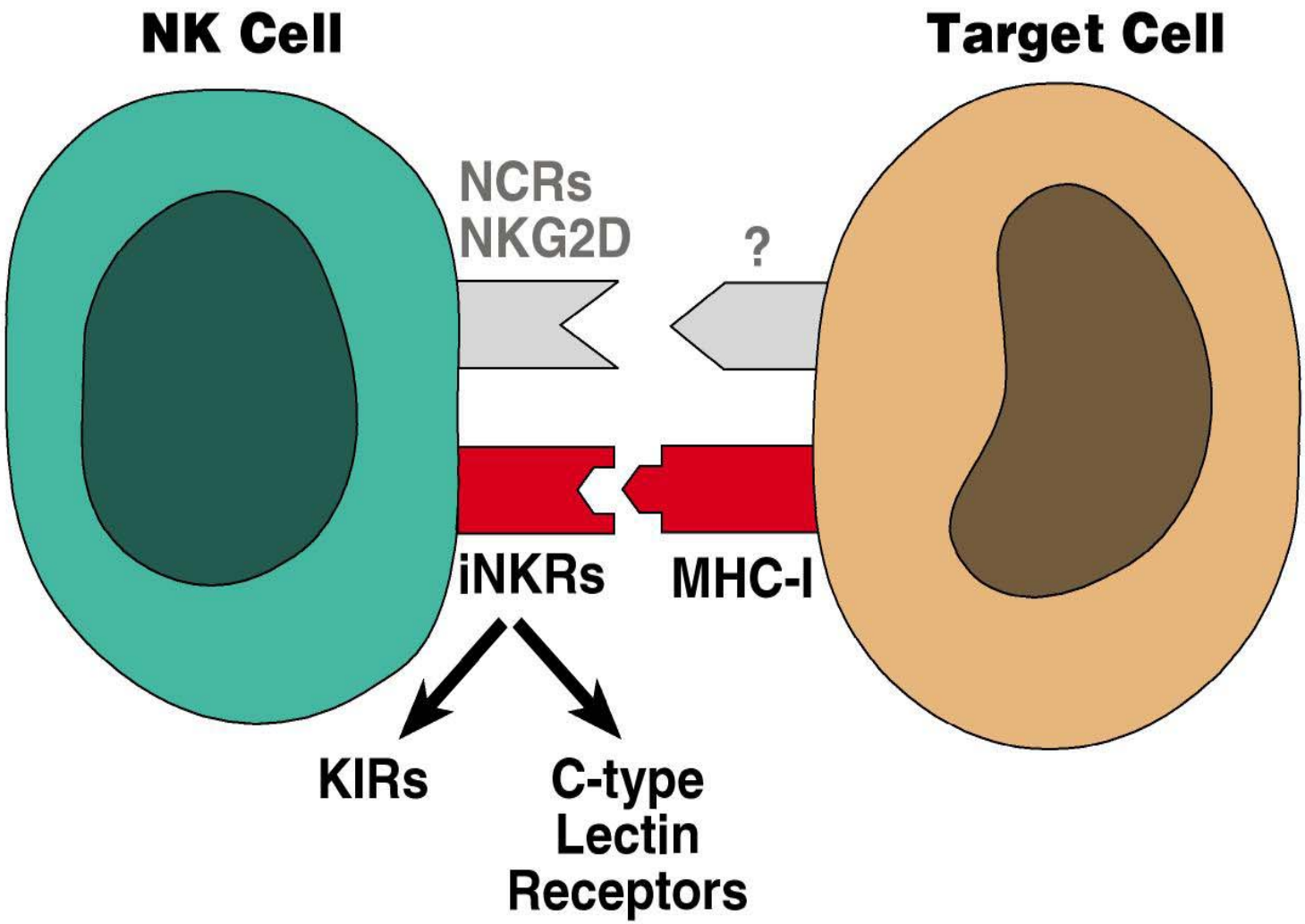


Receptors	NKp46	NKp30	NKp44	NKG2D	2B4	NTB-A	DNAM-1	NKp80
(Signaling polypeptides)	(ζ/γ)	(ζ/γ)	(DAP12)	(DAP10)	(LAT)			

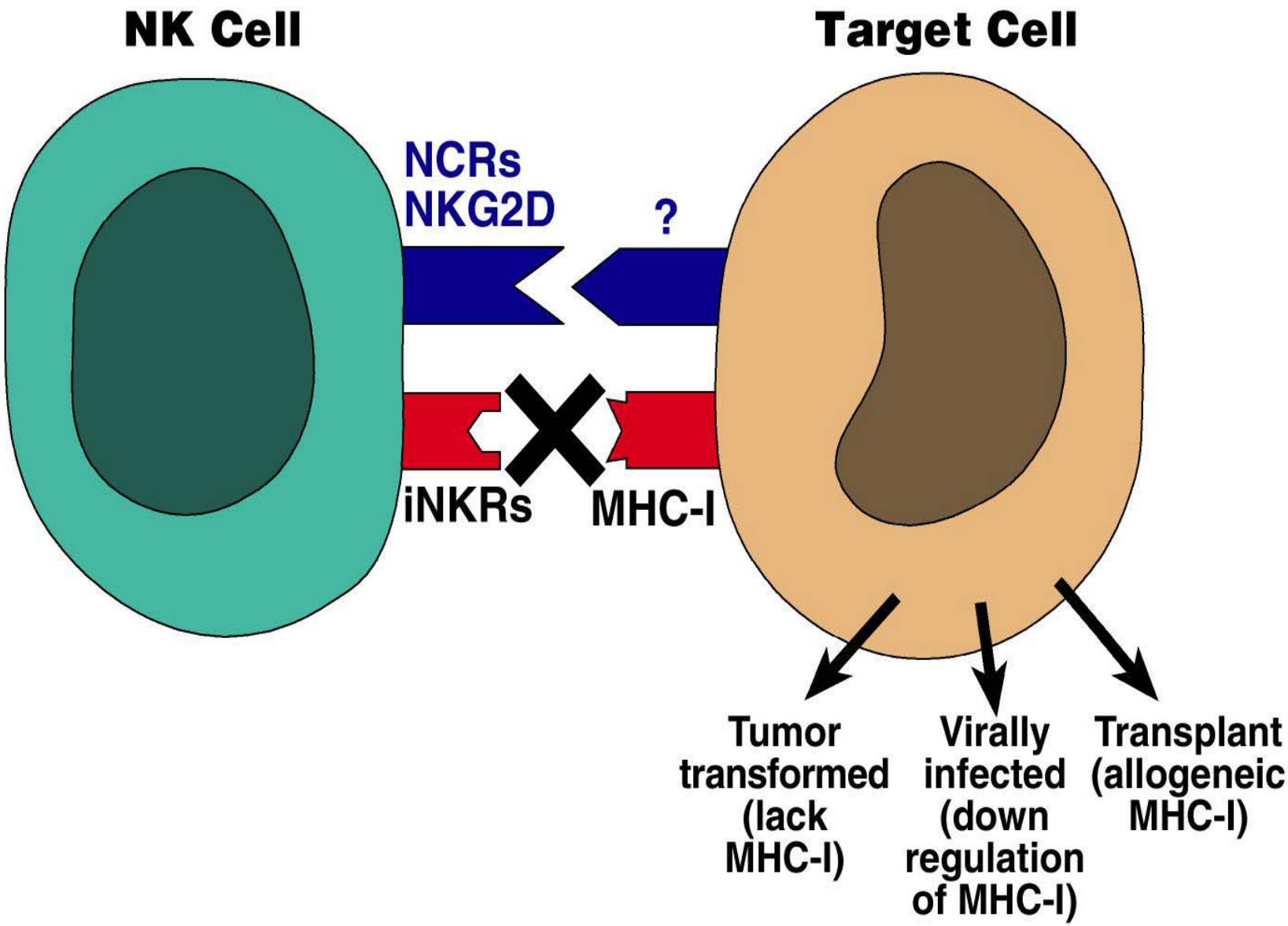
NK Cell Inhibitory Receptors



NK Cell Function



NK Cell Function



PNAS
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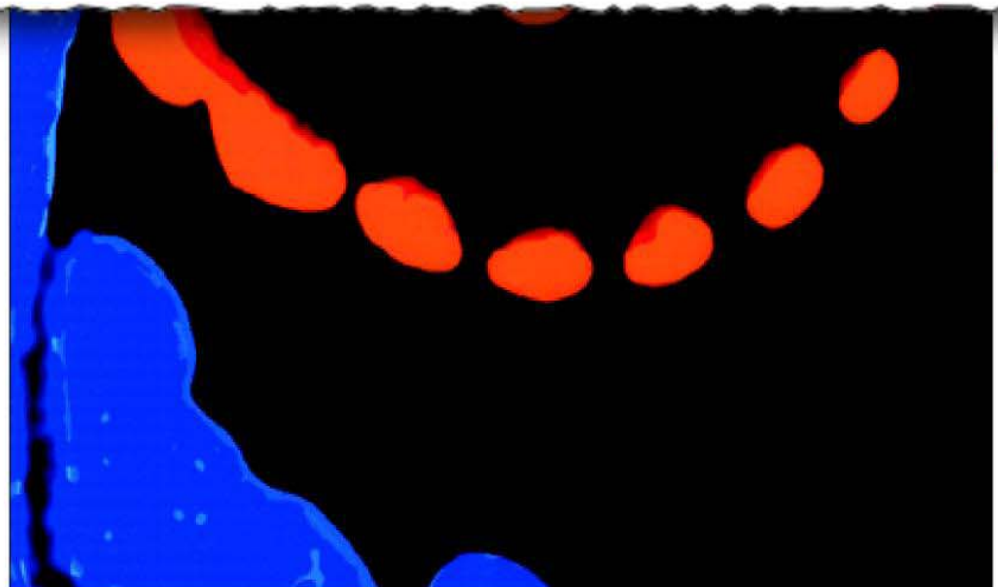
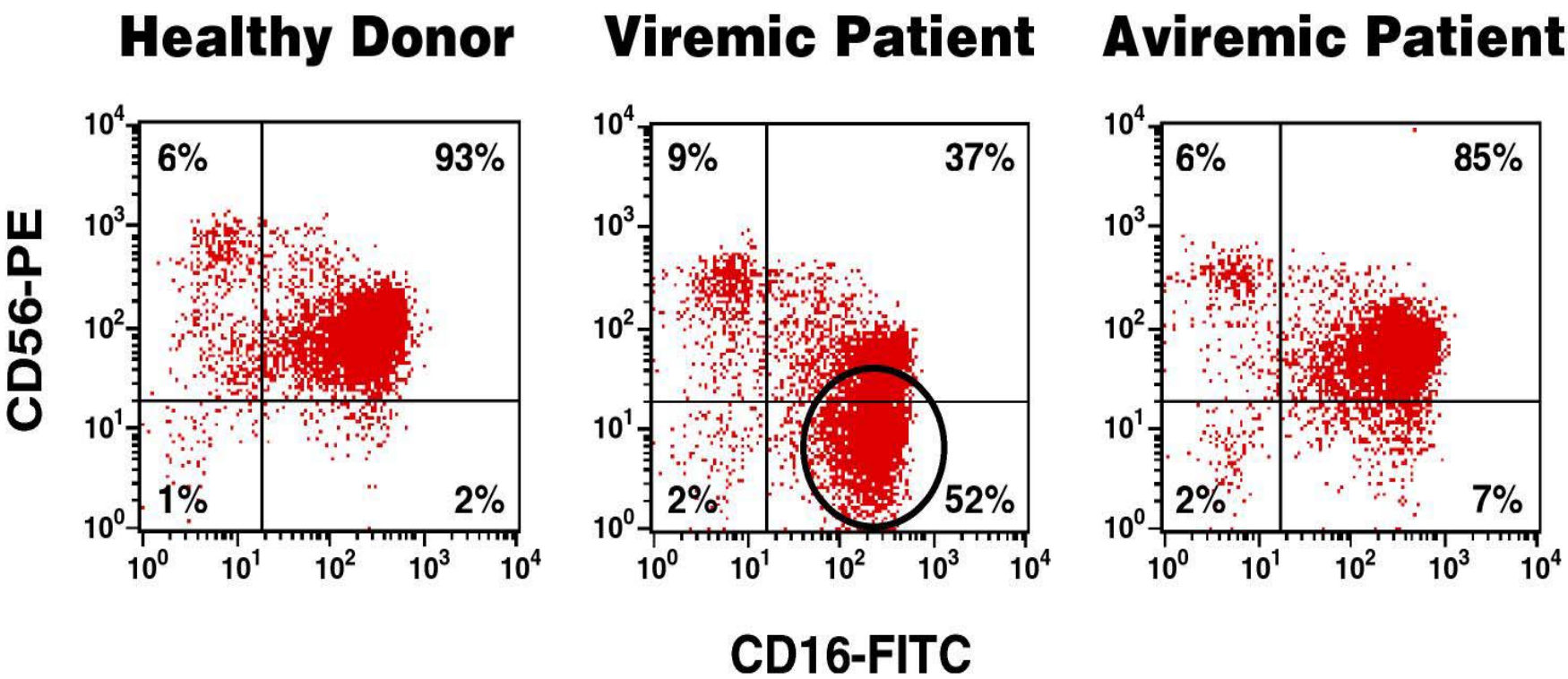
Natural Killer Cells in HIV-1 Infection: Dichotomous Effects of Viremia on Inhibitory and Activating Receptors and Their Functional Correlates

Domenico Mavilio et al

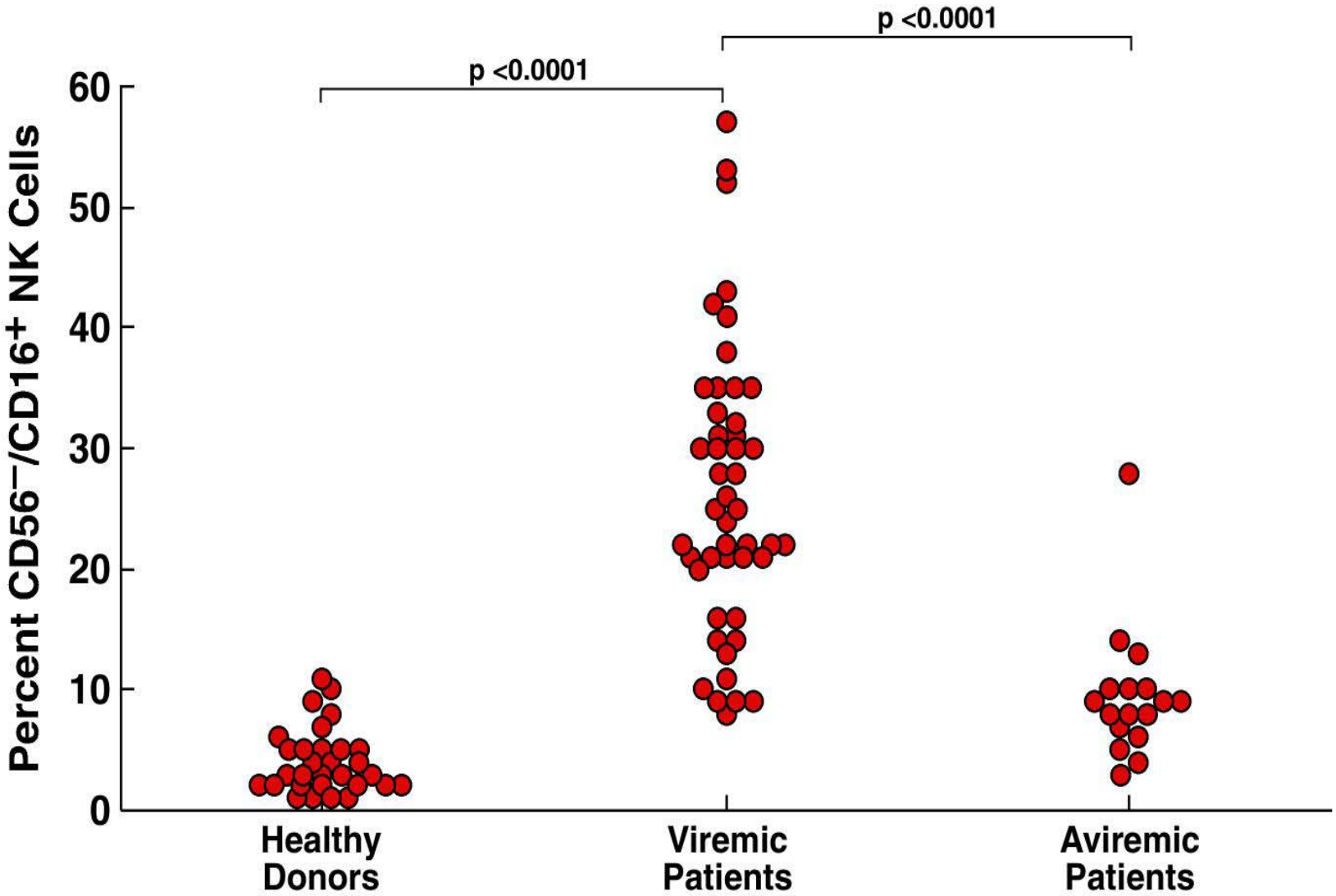


- HIV viremic patients manifest:
  - Decrease in activating receptors (NCRs)
  - Normal or increase in inhibitory receptors

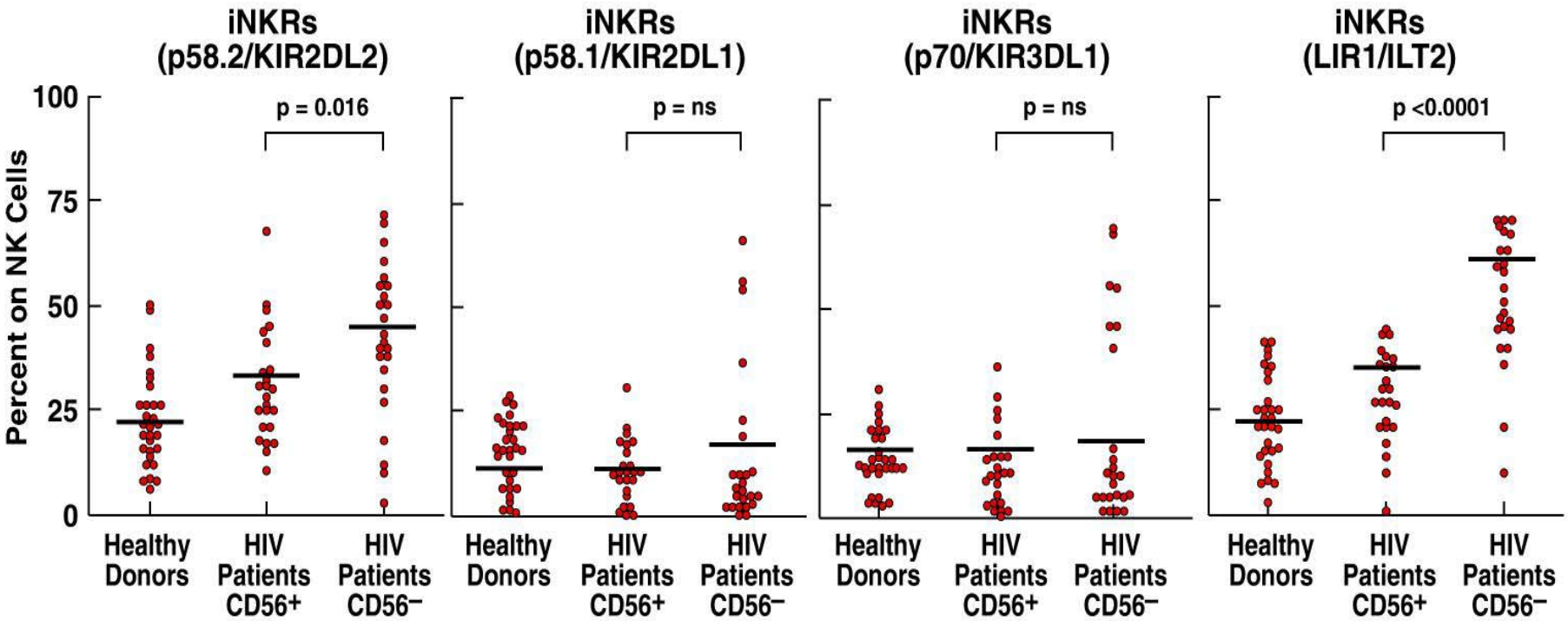
Enrichment of CD56⁻/CD16⁺ NK Cells in HIV-1 Viremic Patients



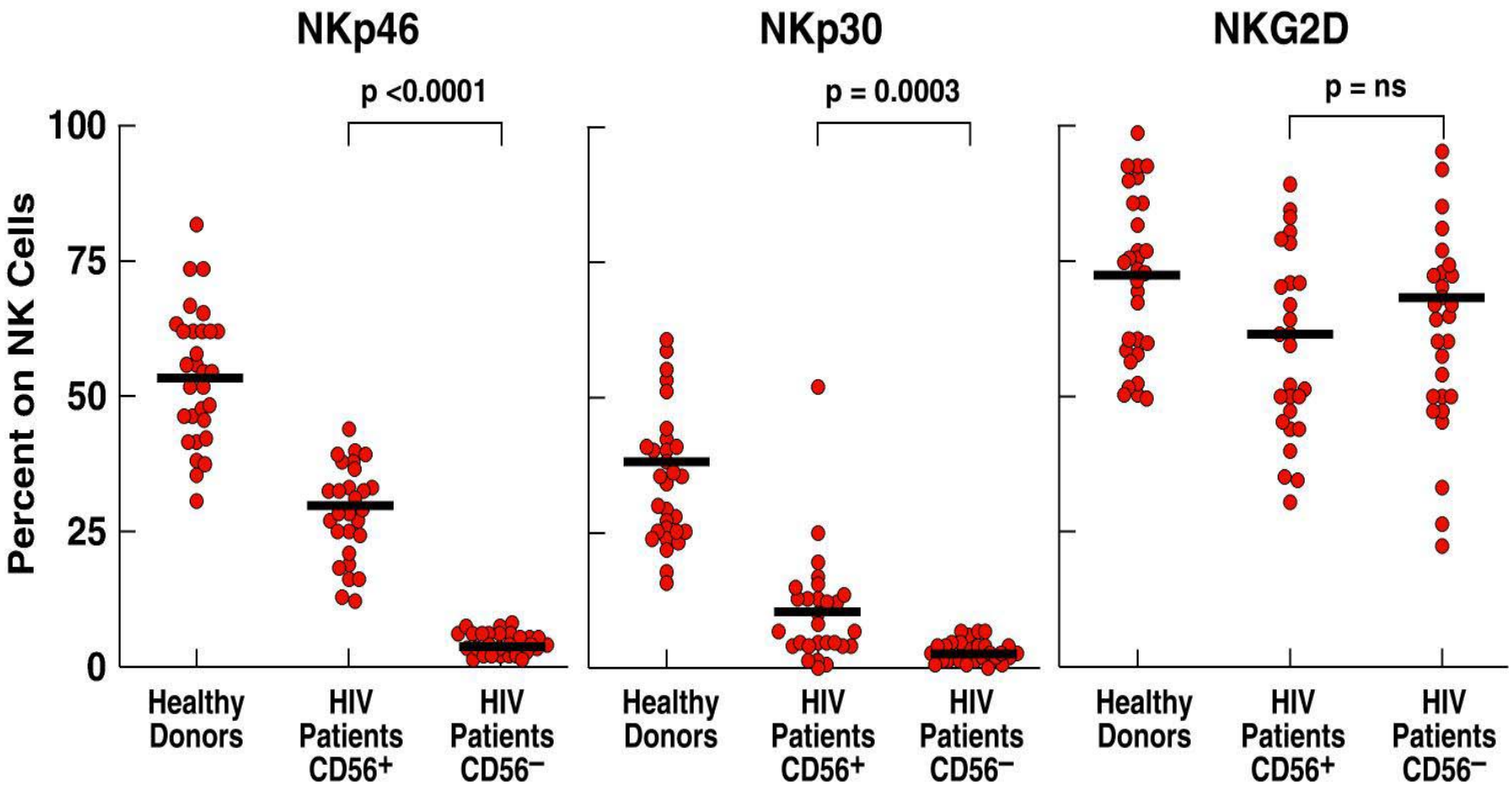
The CD56⁻/CD16⁺ NK Cell Subset is Enriched in HIV-Viremic Patients



Inhibitory NK Receptors are Either Conserved or Increased in CD56^{-} NK Cells of HIV-Viremic Patients



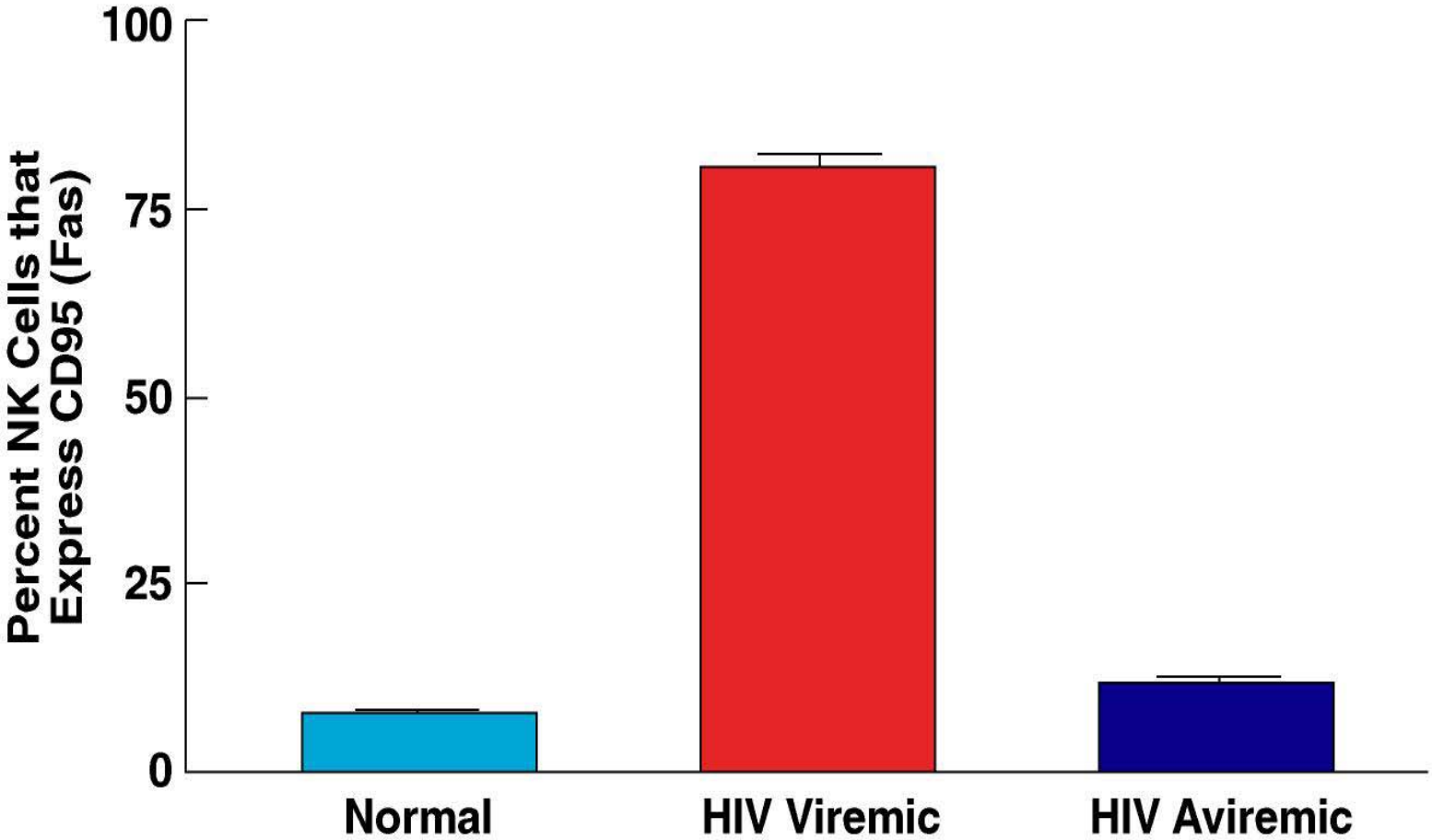
Activating NK Receptors are Decreased in CD56^{-} NK Cells of HIV-Viremic Patients



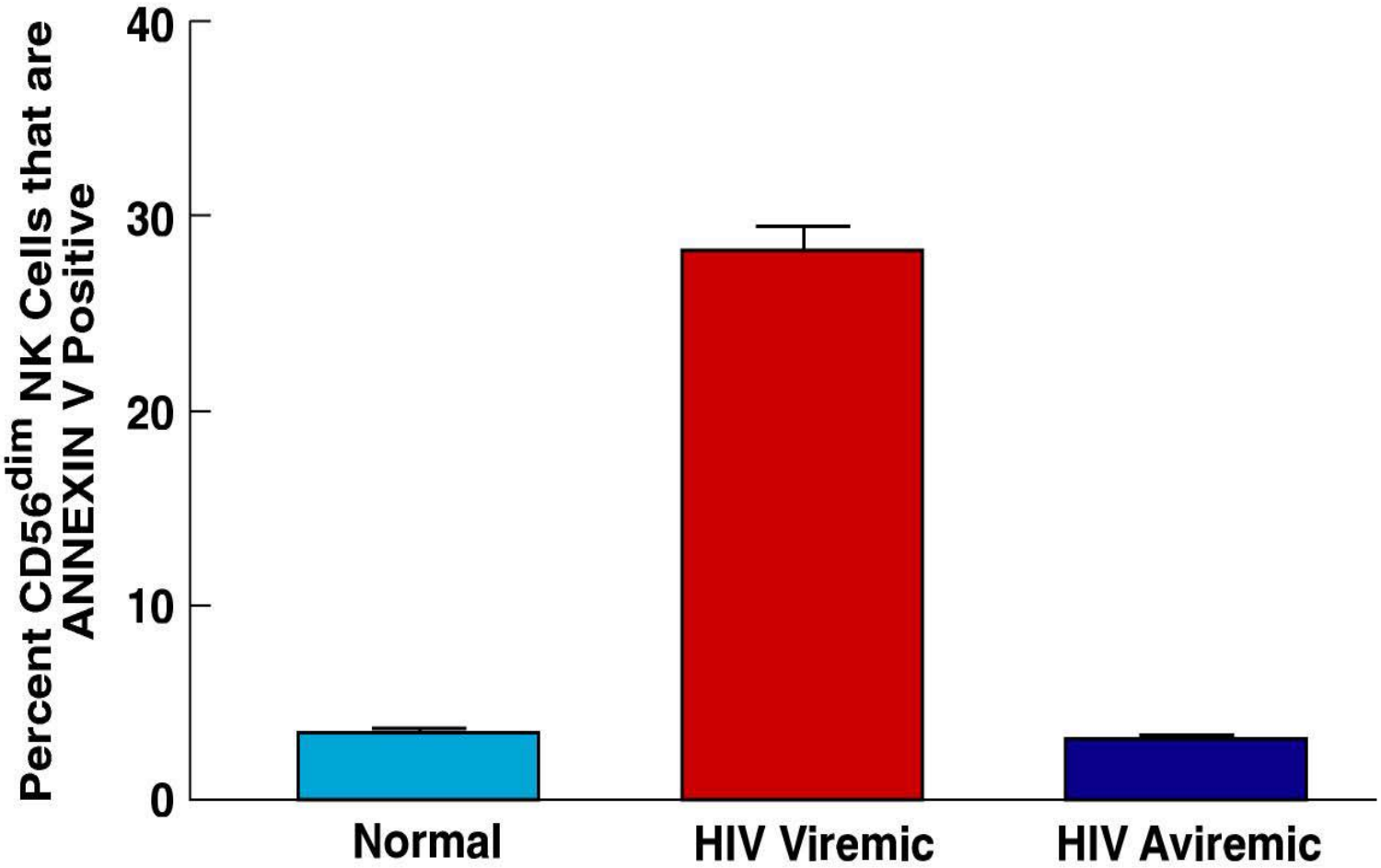
Effect of HIV Viremia on CD56- NK Cells

- Decreased production of perforin and granzyme
- Decreased secretion of TNF-α and interferon-γ

NK Cells from HIV Viremic Individuals Express Fas on their Surface at a Significantly Higher Level than do NK Cells from Normal and HIV-Aviremic Individuals



NK Cells from HIV-Viremic Individuals Undergo Increased Apoptosis upon Exposure to sFasL *in vitro*

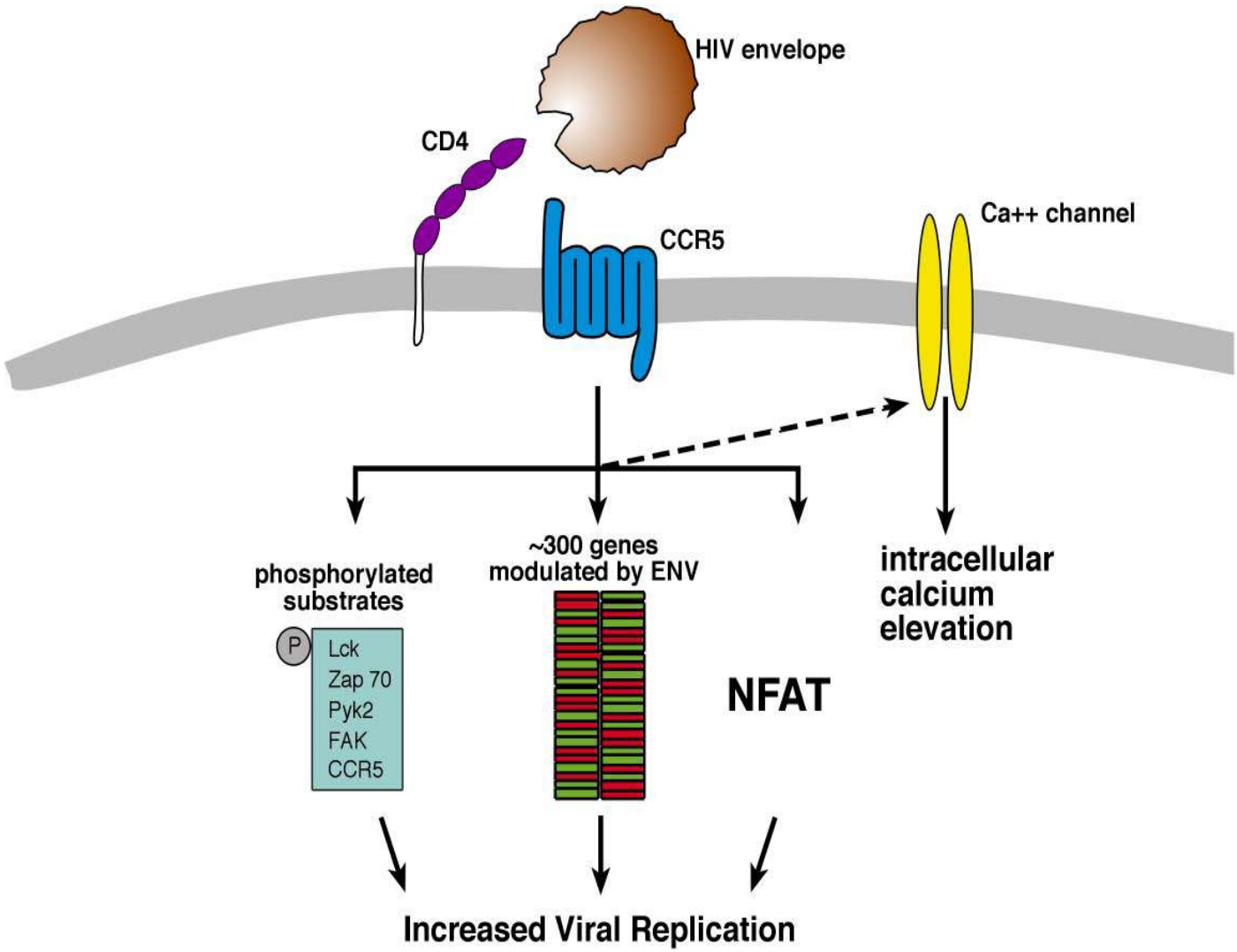


Effect of HIV Viremia on NK Cell Phenotype and Function

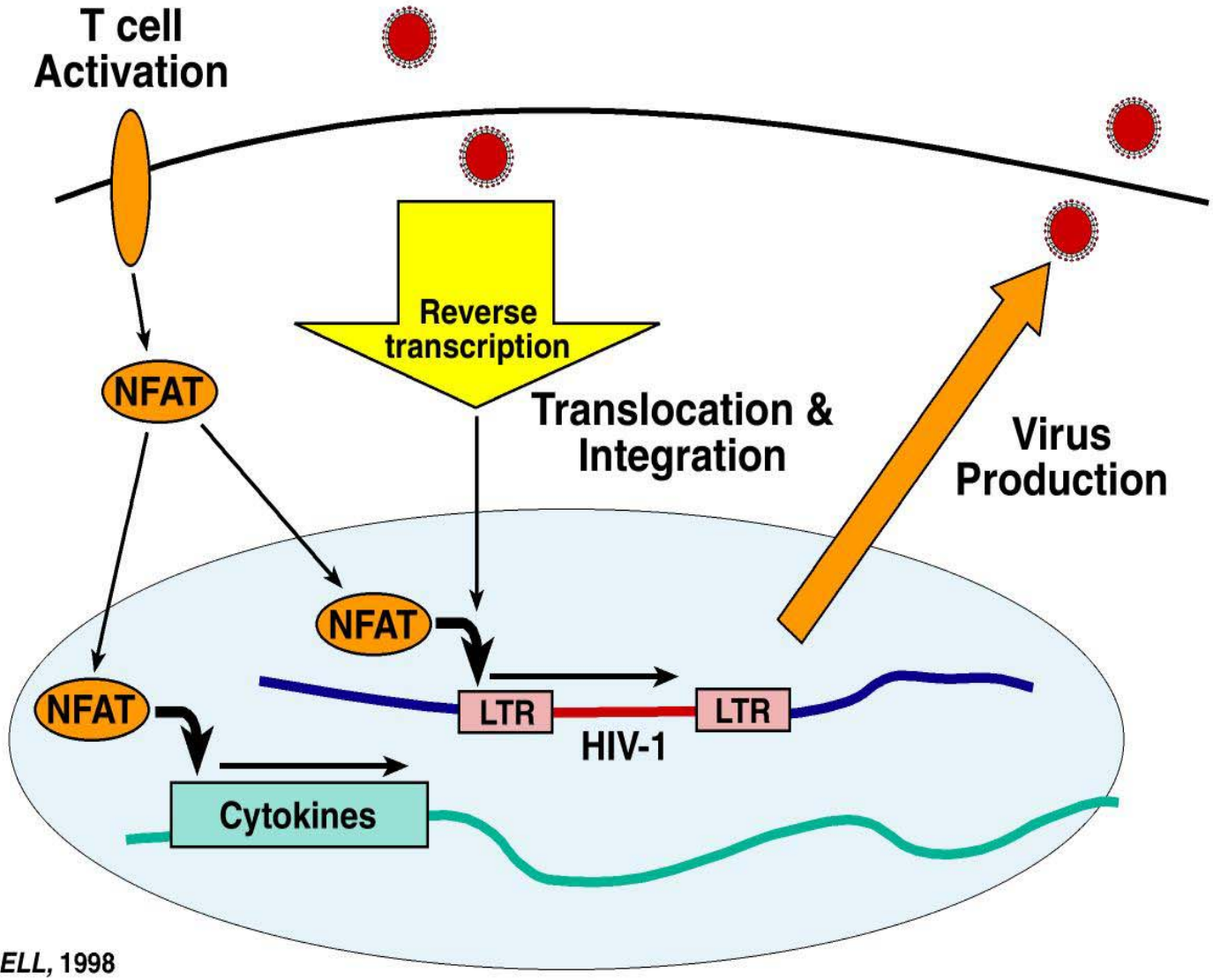
- HIV-viremic patients manifest a dichotomy of expression of NK cell receptors: inhibitory receptors are maintained or increased while activating receptors are decreased.
- This dichotomy is reflected in decreased cytotoxic function as well as a decrease in production of perforin and granzyme and a decrease in secretion of TNF-α and IFN-γ.
- Viremia is associated with an enrichment of $CD56^{-}/CD16^{+}$ subset of NK cells. The defects in receptor phenotype and function are concentrated within this abnormal subset.
- NK cells from viremic patients express increased levels of Fas on their surface and the $CD56^{dim}/CD16^{+}$ subset responds to sFasL with increased apoptosis.

Direct Effects of HIV Envelope on Immune Cell Function

Role of HIV Envelope in the Induction of Aberrant Signal Transduction

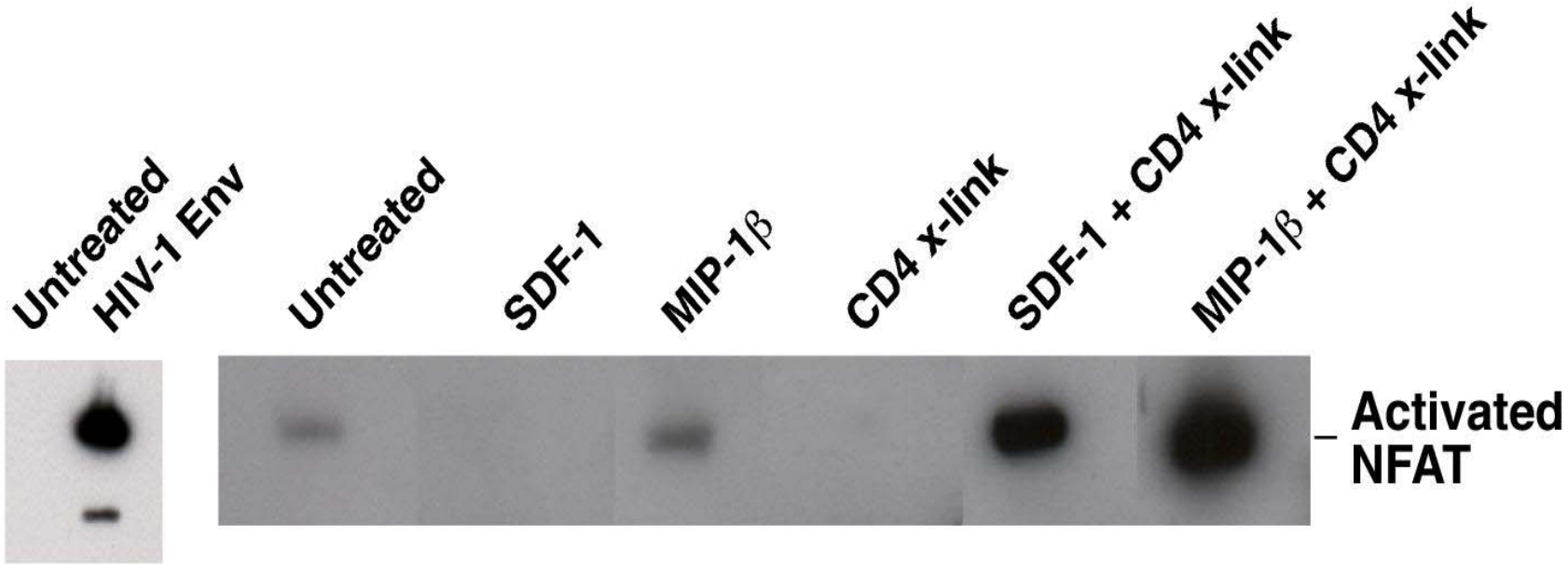


The Role of NFAT in HIV Life Cycle

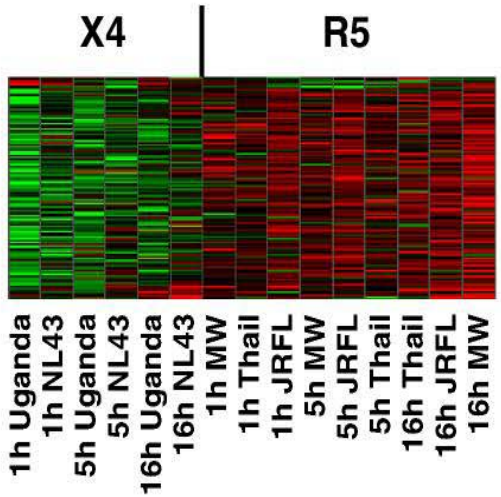


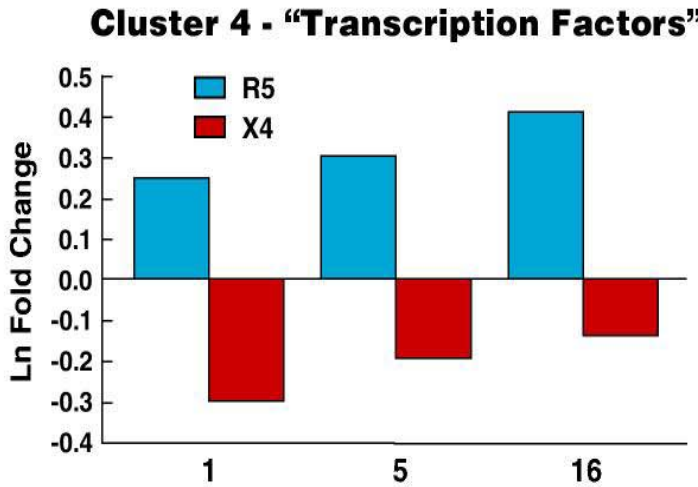
Kinoshita et al., *CELL*, 1998

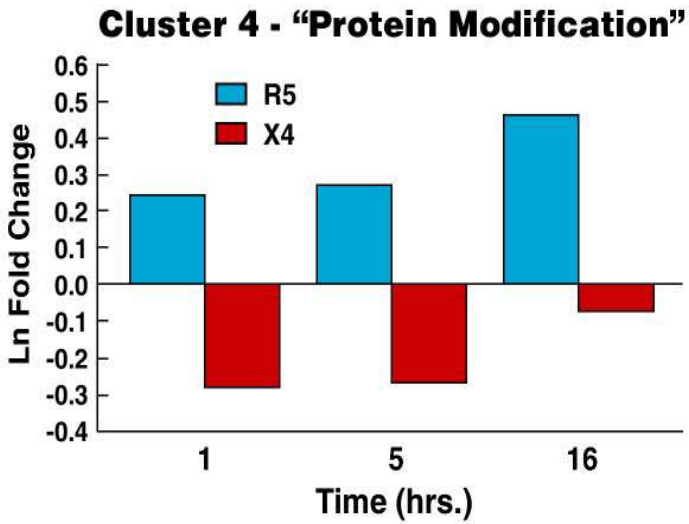
NFAT Activation through HIV Envelope Requires Coordinate Signaling through CD4 and Coreceptor

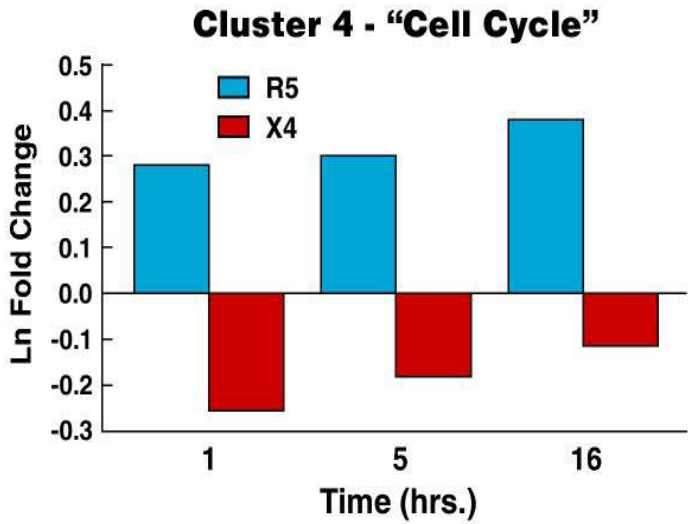


Categories of Genes Differentially Modulated by R5 vs X4 Envelopes





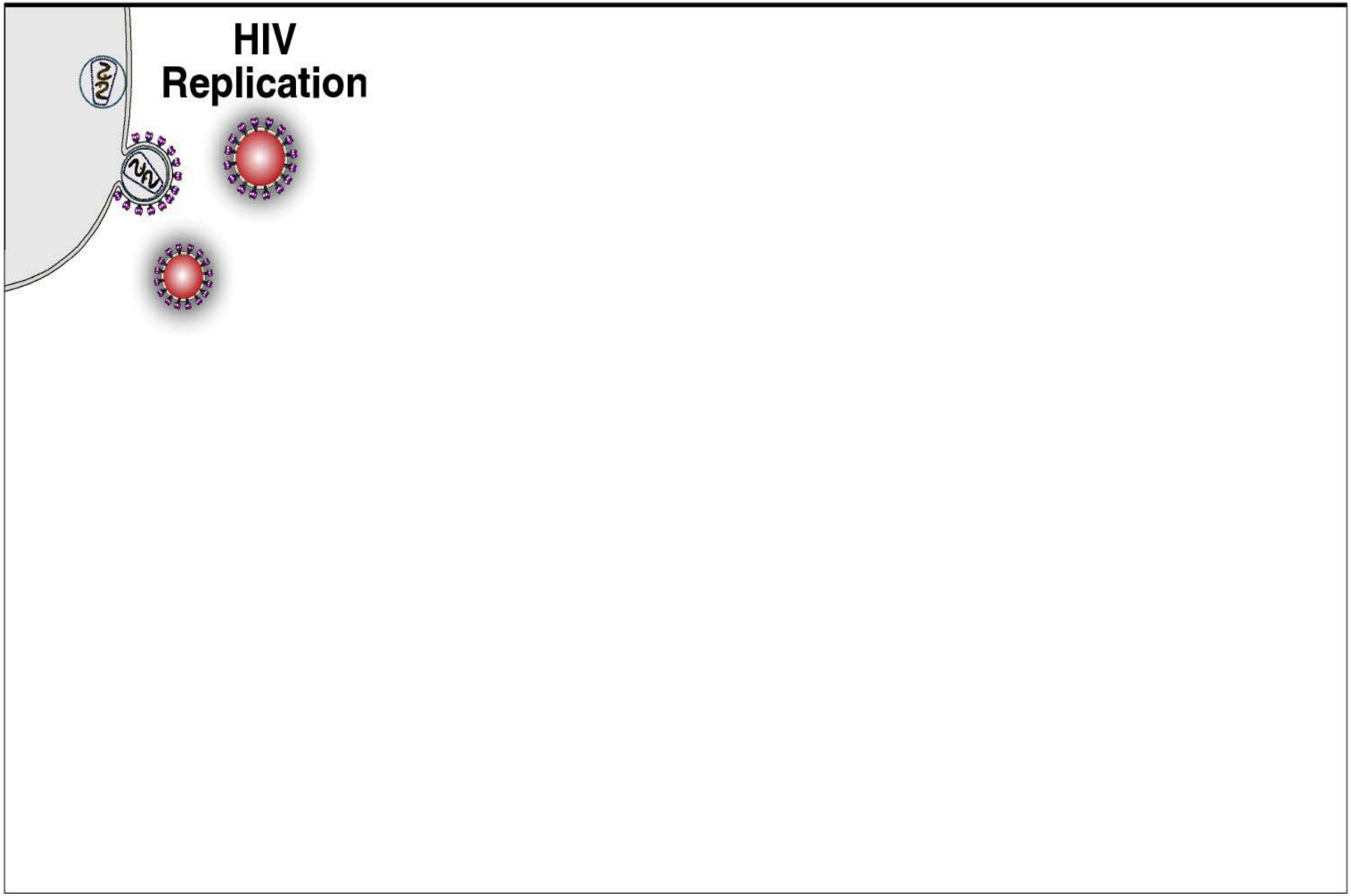




Conclusions

- HIV envelope modulates PBMC gene expression that is associated with virus replication and the secretion of cytokines involved in immune activation.
- This induction of gene expression requires coordinate signaling through CD4 and the respective co-receptor.
- Envelopes derived from X4 versus R5 viruses can differentially induce the expression of genes associated with transcription factors, cell cycle, and protein modification.
- Thus, HIV envelope-induced cell signaling may play an important role in the propagation of virus replication and the state of aberrant immune activation.

Pathogenic Mechanisms of HIV Disease



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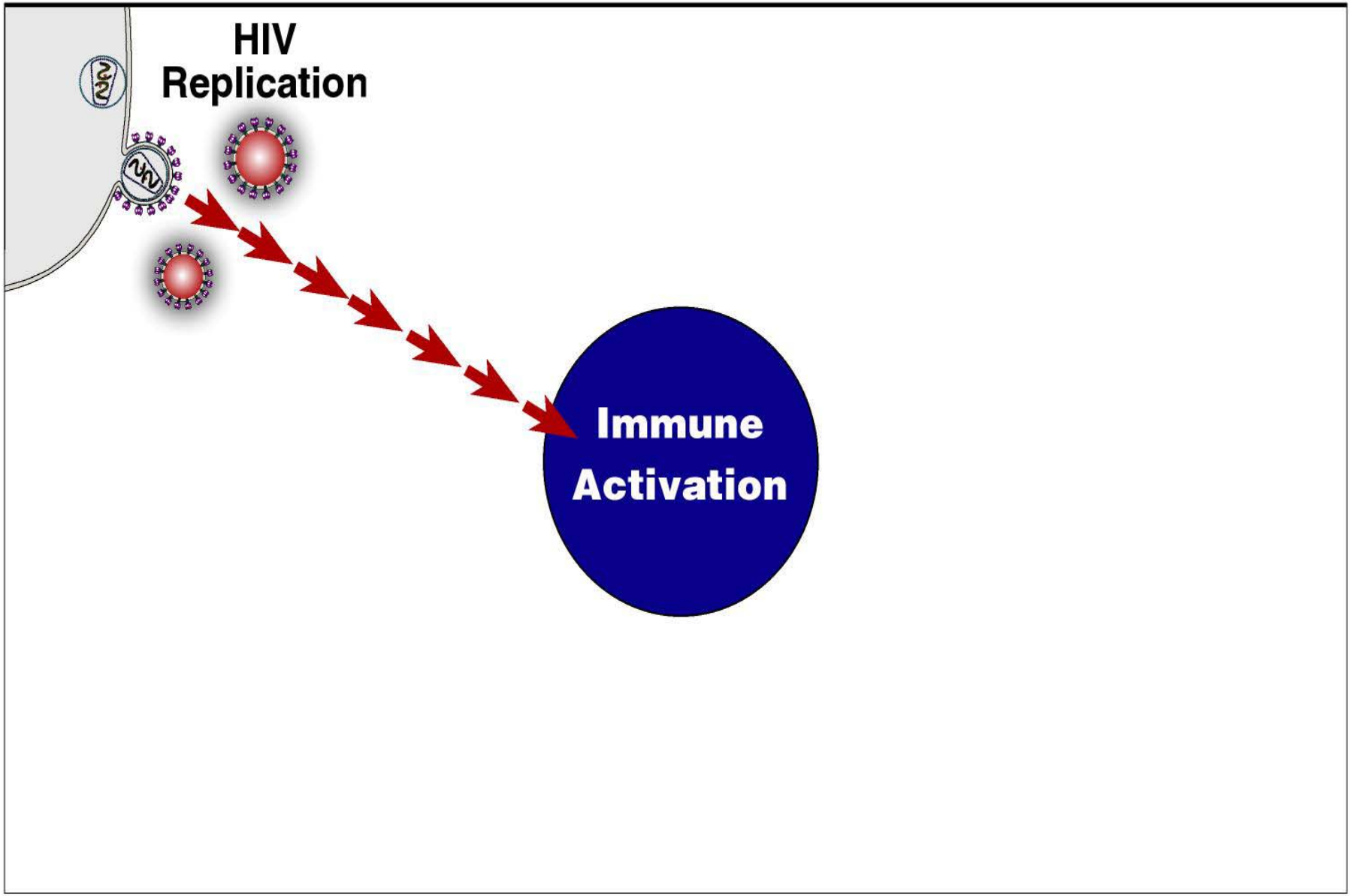

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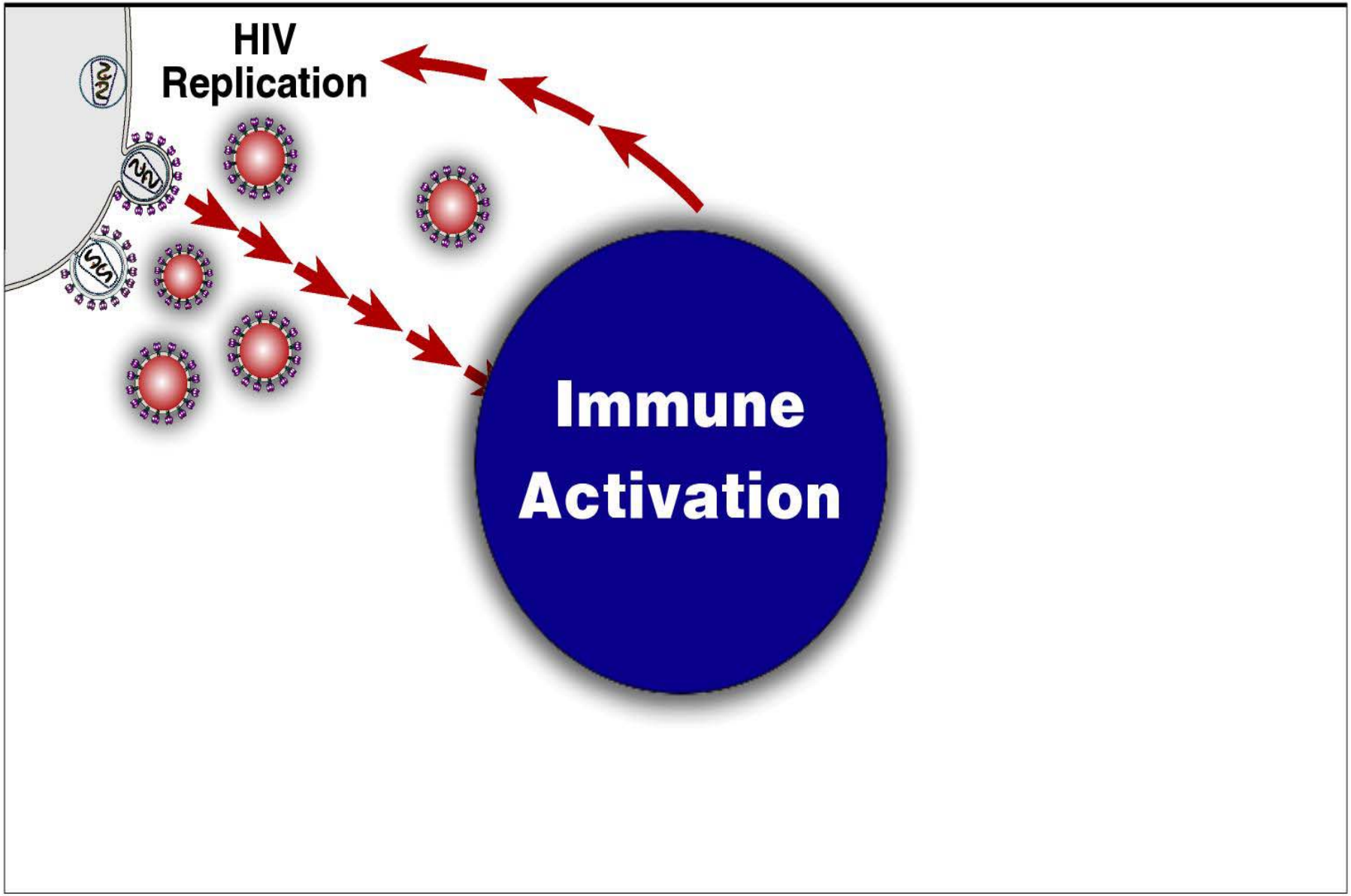

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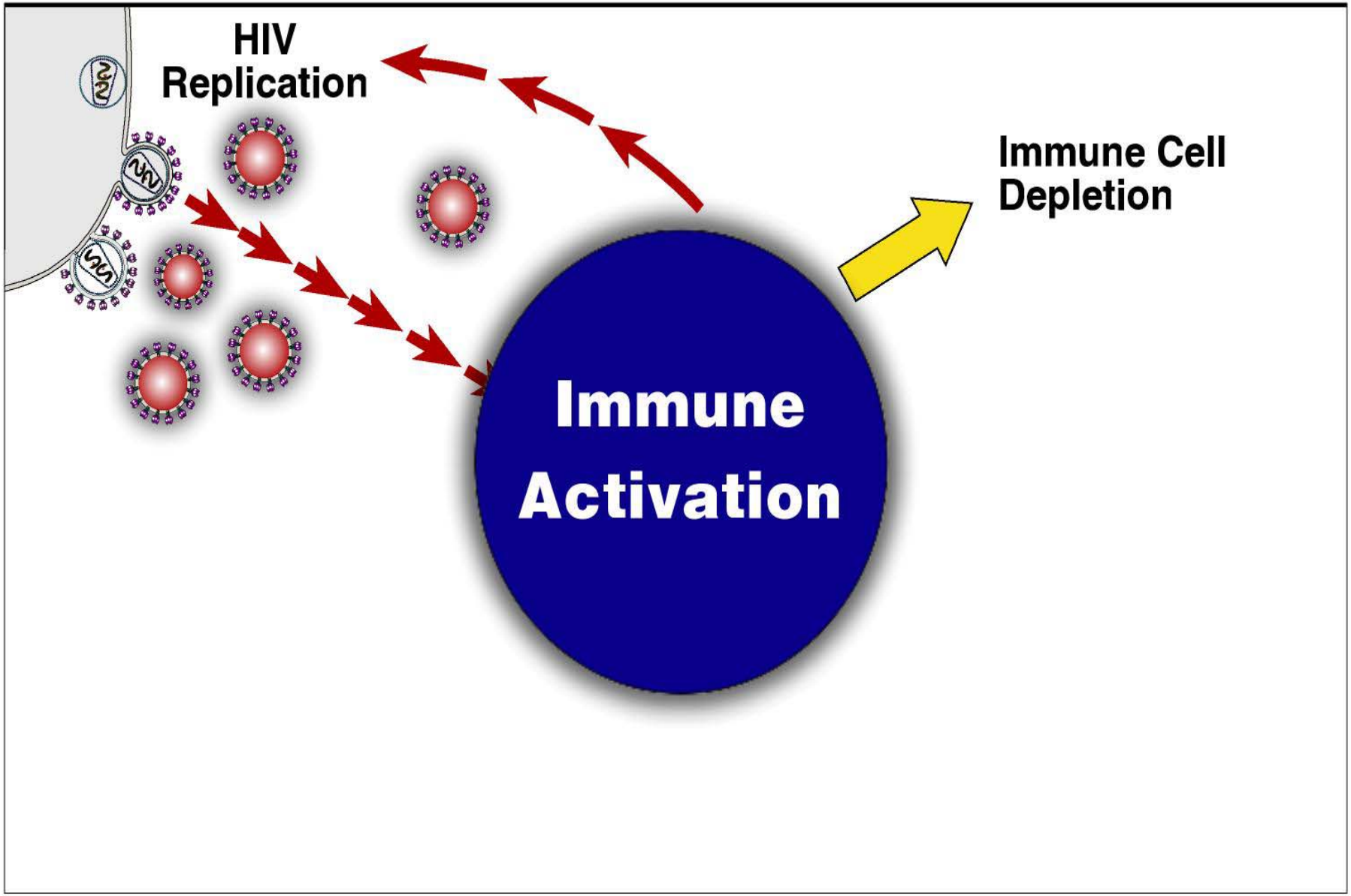

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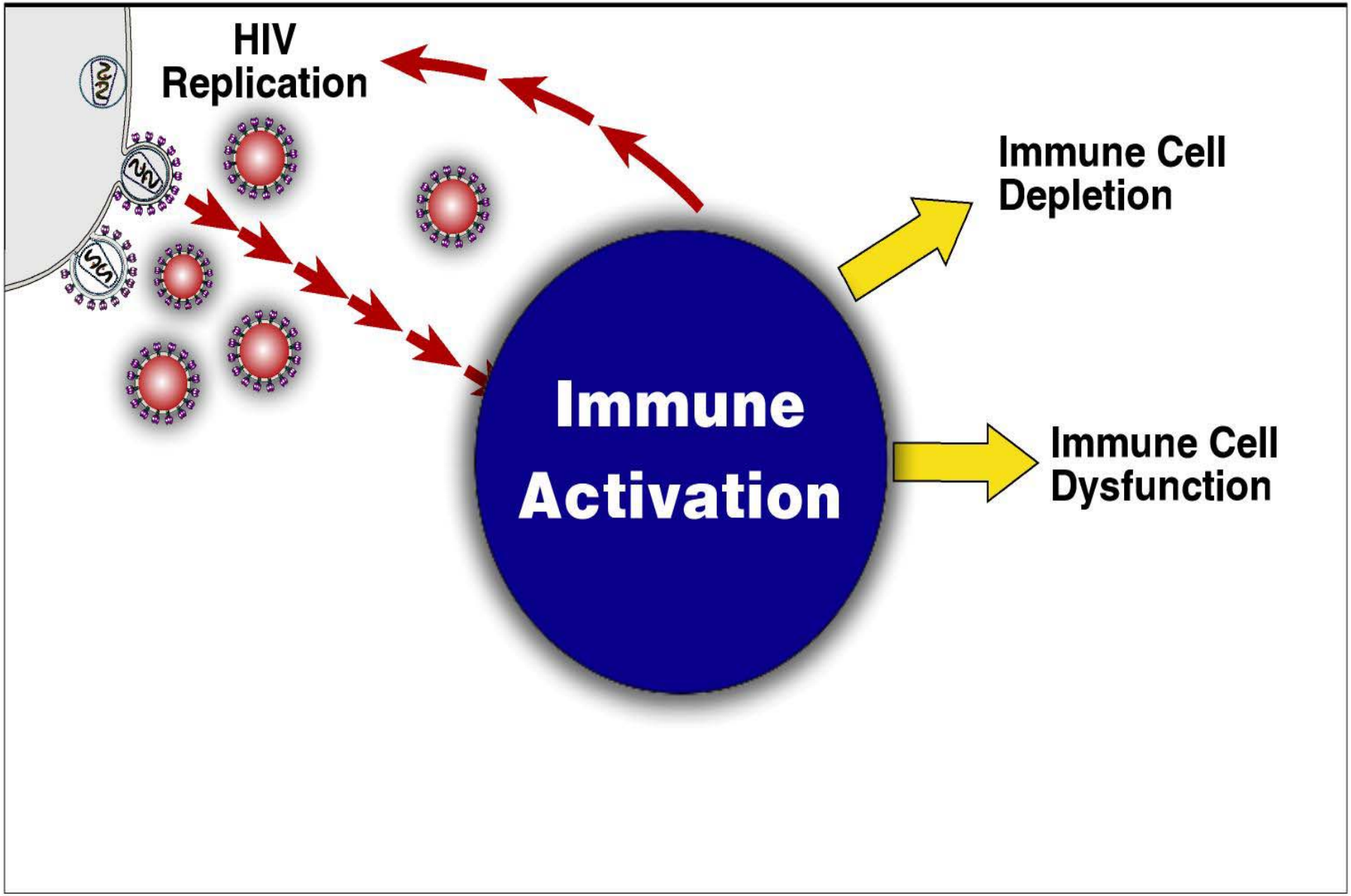

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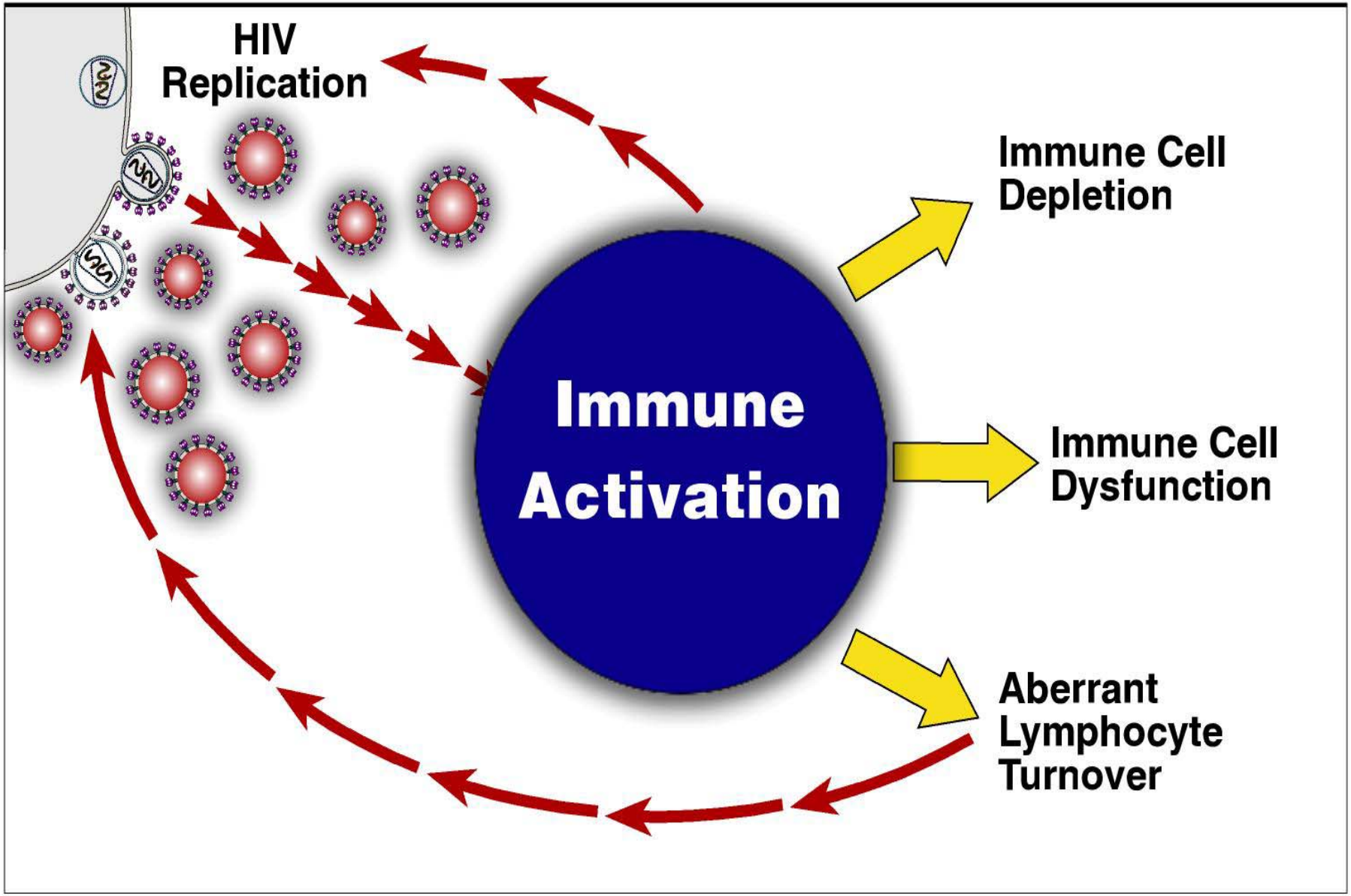

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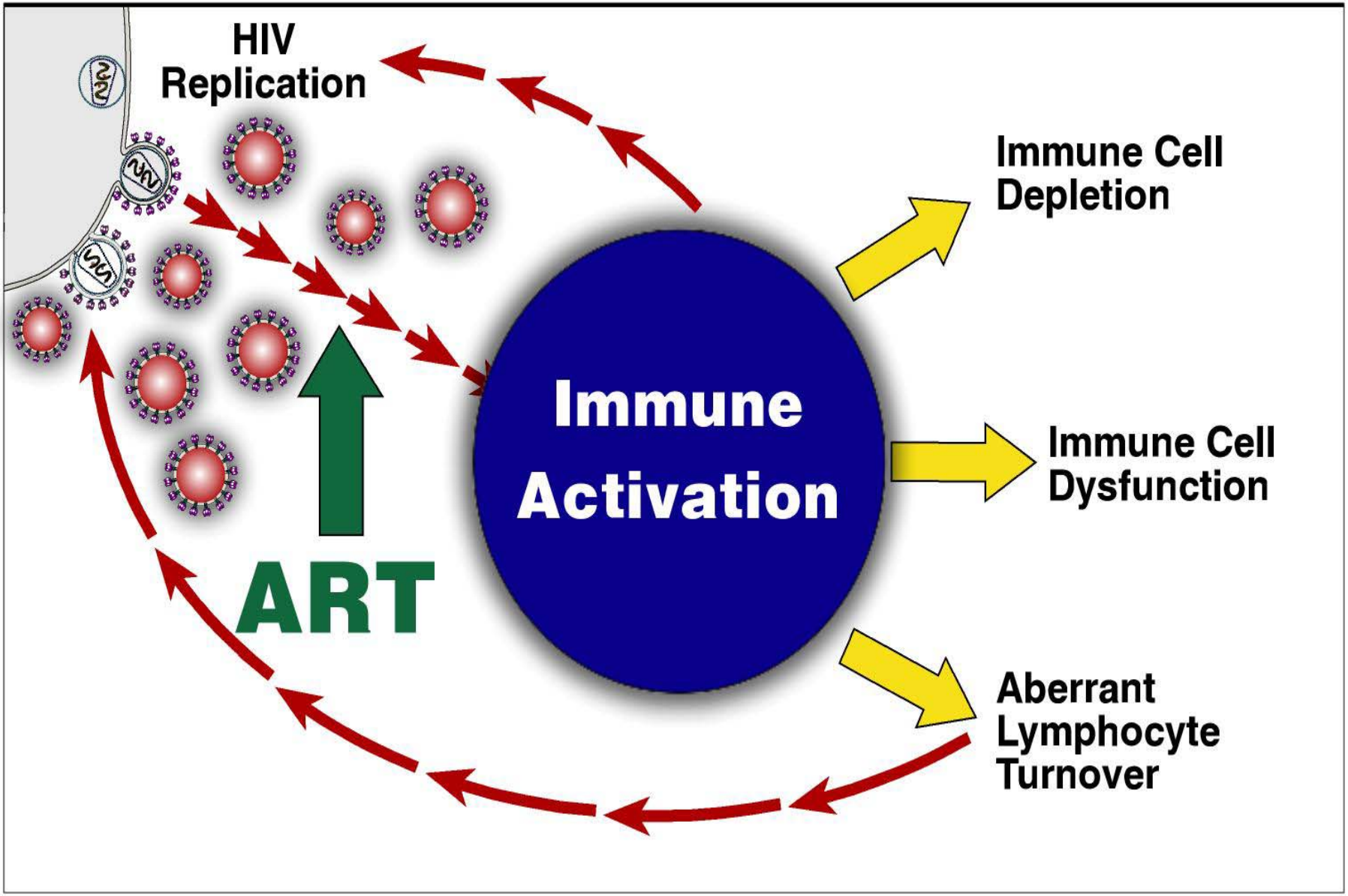

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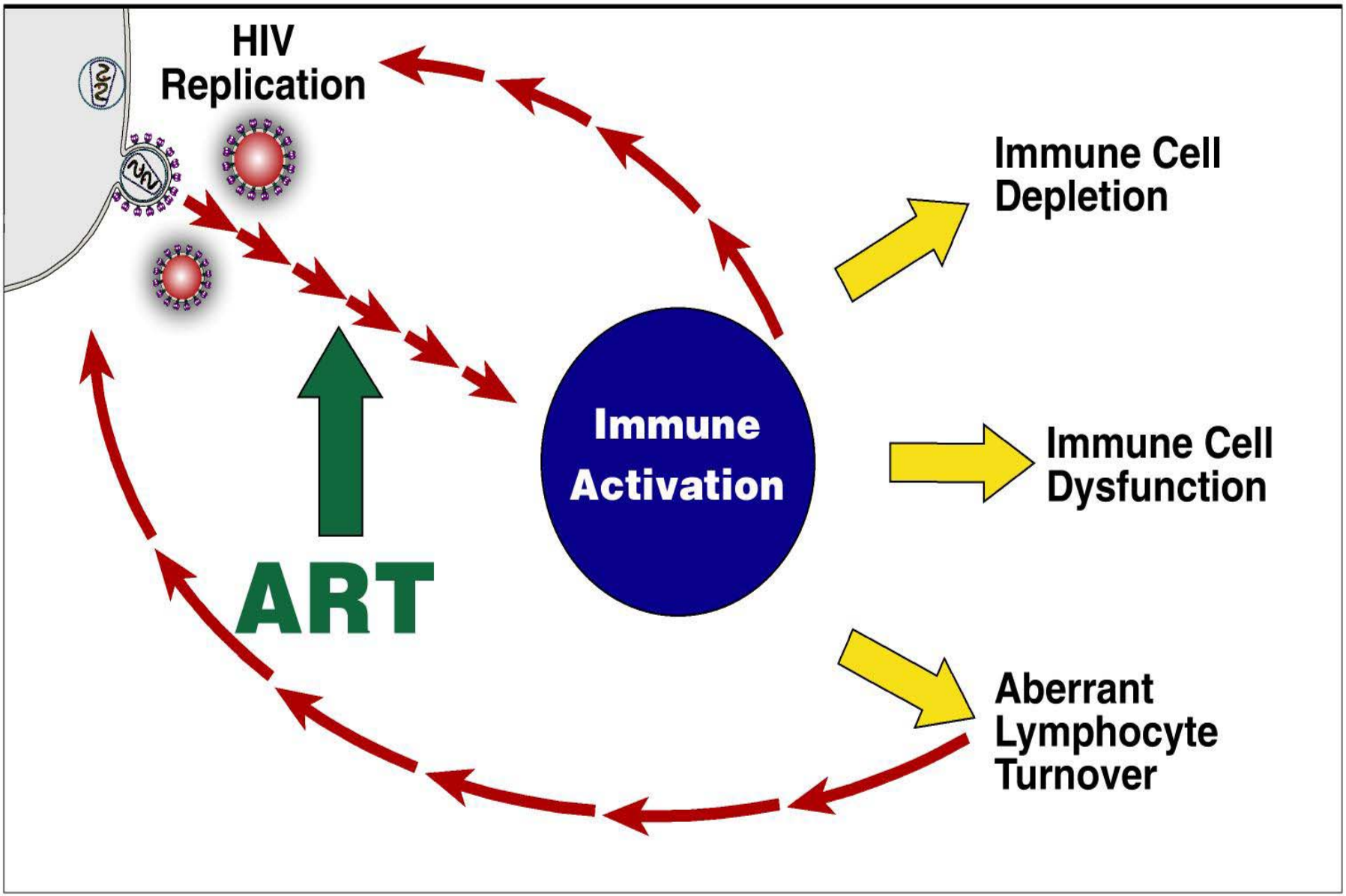

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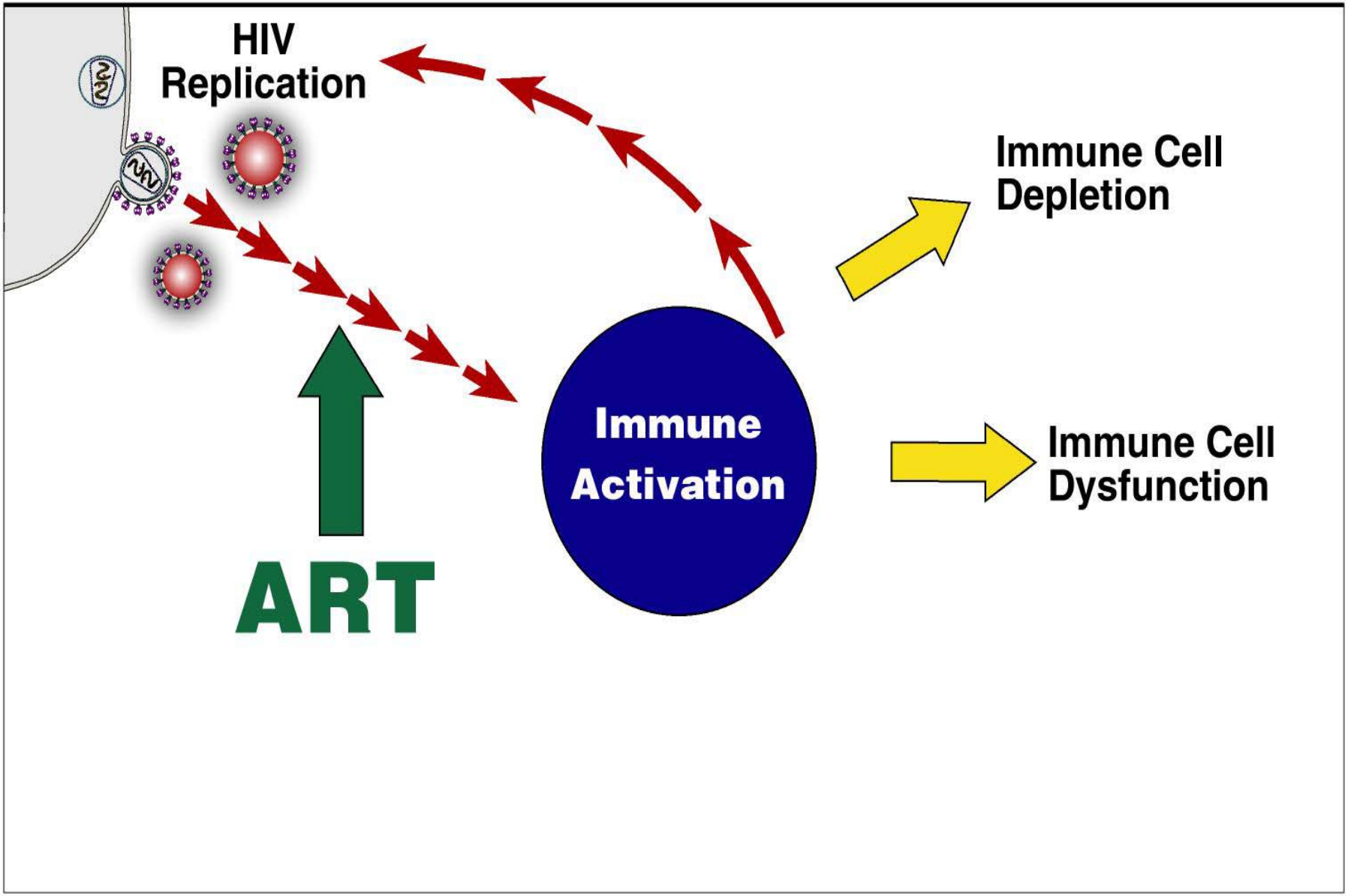

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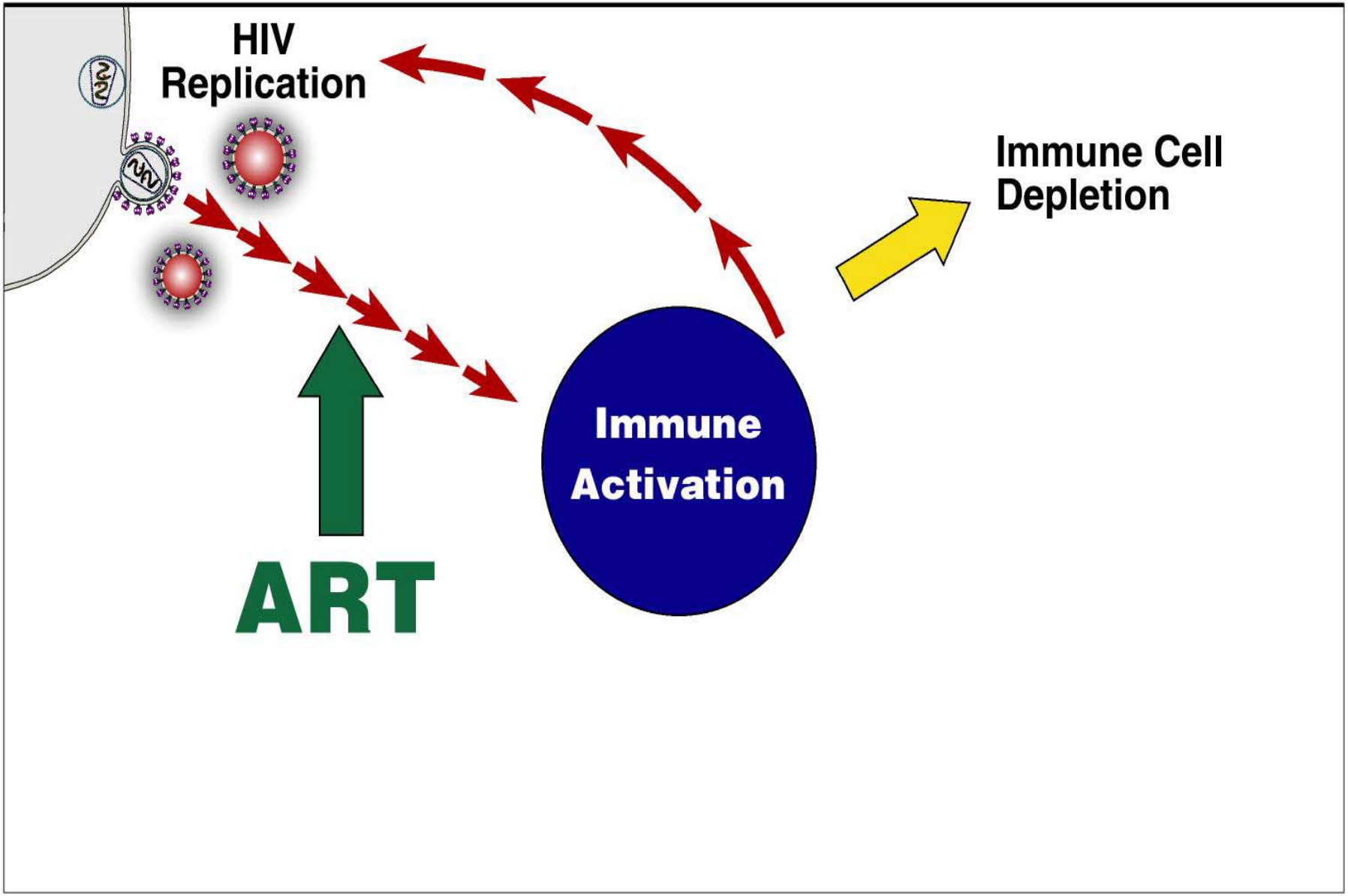

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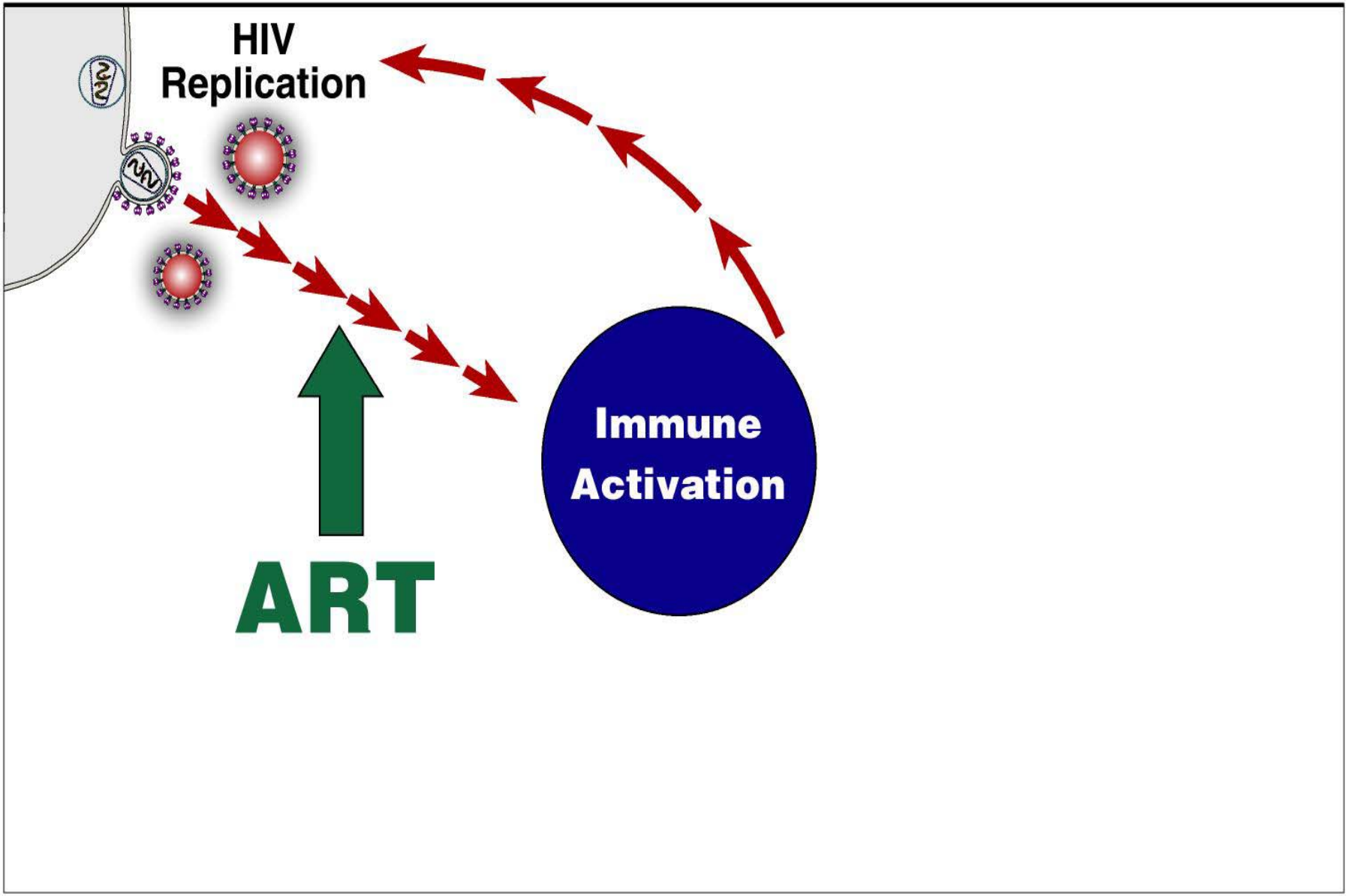

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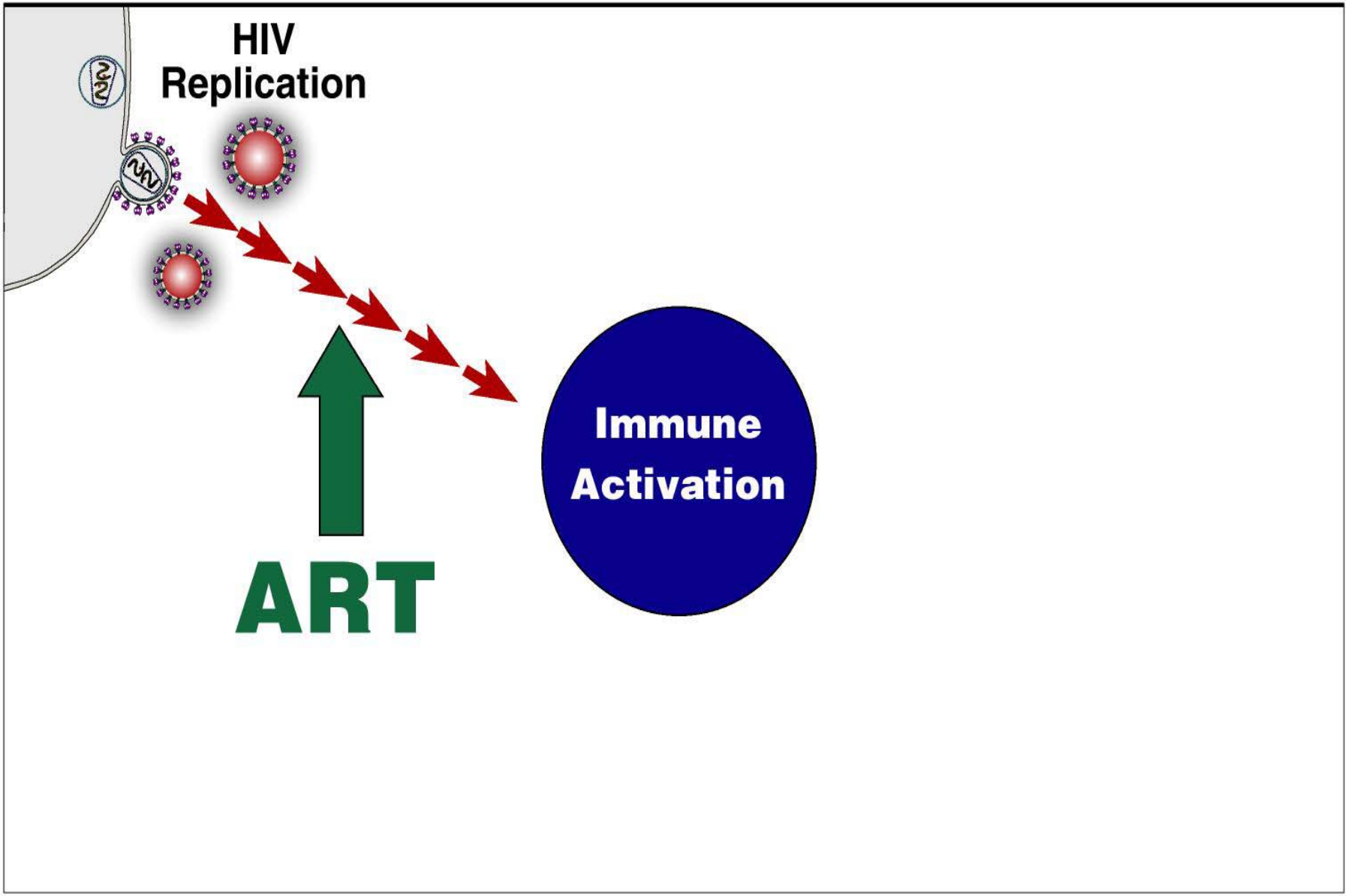

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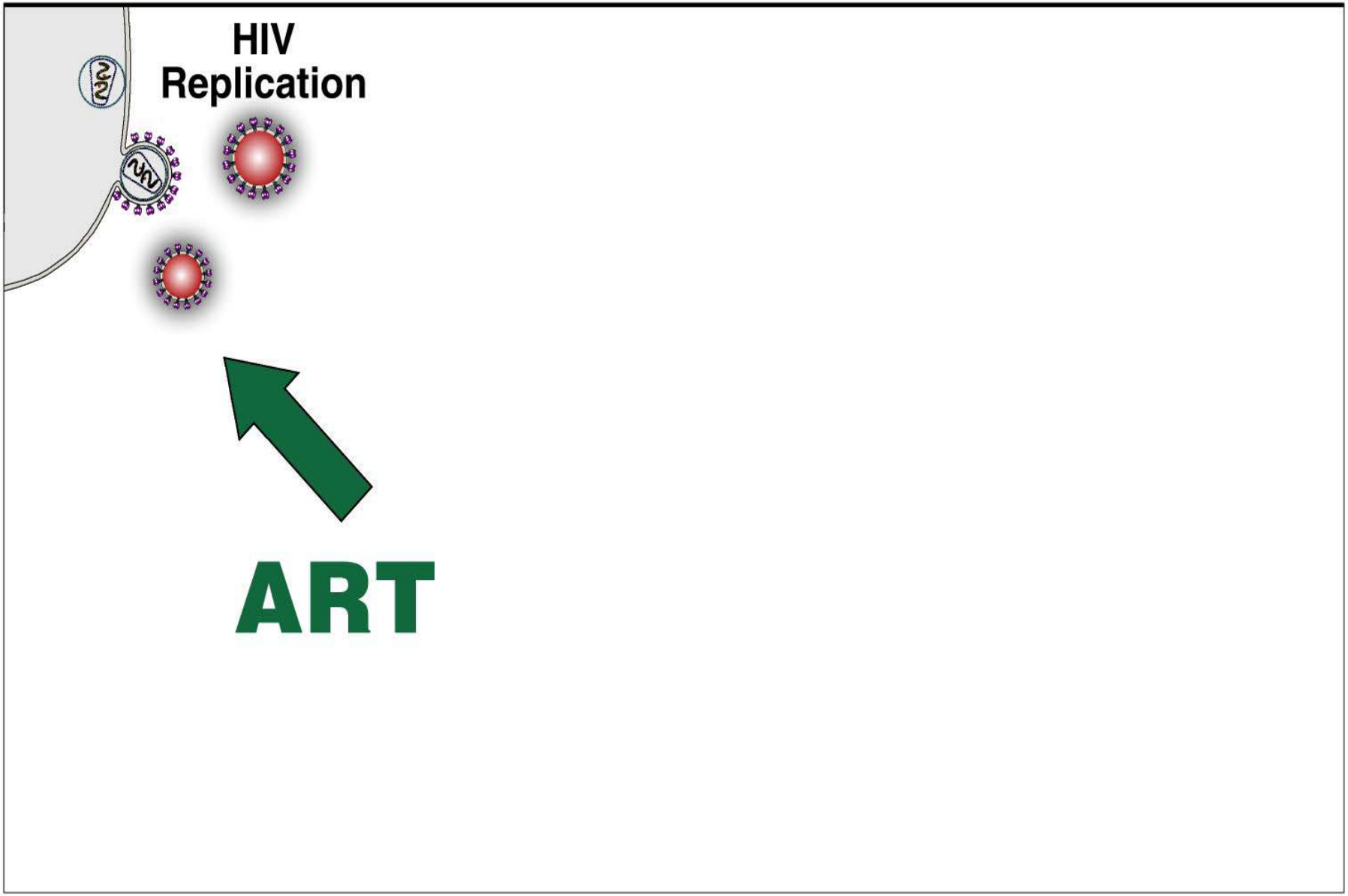

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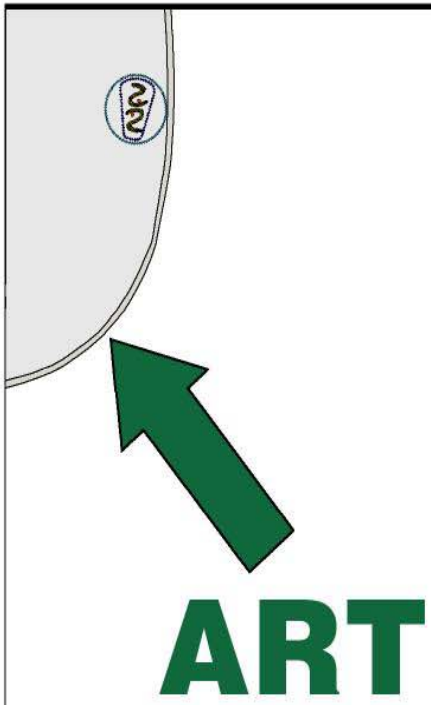

ART

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